

P Element-Induced, X-Linked Lethal Mutations Causing
Melanotic Tumors in Drosophila melanogaster

by

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CORRIGENDUM

The cytological map position of air8 as displayed in
Figure 3B (page 40) should be extended to include 7C9
(ie. 7C4-6; 7C9)

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LIST OF ABBREVIATIONS/DESCRIPTIONS

A	Adults
AM	Anterior midgut
<u>air</u>	Aberrant immune response mutation
<u>Binsn</u>	X-chromosome balancer
BH (Bh)	Brain hemispheres
DV	Dorsal vessel
EL	Early larvae, 1st and 2nd instar
EP	Early pupae
Eye/an	Eye-antennal imaginal disc
<u>FM7</u>	X-chromosome balancer
<u>Gar</u>	Garland cells
Gc	Gastric caeca
ID	Imaginal discs
In	Intestine
LG	Lymph gland lobes
LL	Late larvae, 3rd instar
M cytotype	Permissive cellular environment for P elements
M strain	Drosophila strain devoid of intact P elements
P	Proventriculus
PC	Pericardial cells
P cytotype	Restrictive cellular environment for P elements
Psp	Pseudopupae, combination of larval and pupal characteristics
P strain	Drosophila strain containing intact P elements
RG	Ring gland (corpus allatum, prothoracic gland, corpus cardiacum)
<u>Tum</u> ¹	Tumorous-lethal, X-linked melanotic tumor mutation
<u>tu(1)Sz</u> ^{ts}	Tumor of Suzuki, X-linked melanotic tumor mutation
<u>tu-W</u>	Tumorous-W, autosomal melanotic tumor mutation
<u>VG</u> (Vg)	Ventral ganglion
<u>w</u> ¹	White-1, X-linked marker mutation
<u>y</u> ¹	Yellow-1, X-linked marker mutation

LITERATURE SURVEY

P Element Mutagenesis

The ability to understand the role of a particular gene or its product in the development and/or maintenance of an individual is dependent on clearly defining all the functions and interactions of that gene. One experimental approach is to perturb or abolish the function of a gene and examine what effect this action has on the ability of that individual to complete proper development. Recently, a technique known as P element mutagenesis was developed for use in the genetically well-characterized organism, Drosophila melanogaster. The mutagenesis technique utilizes the transposable genetic elements called P elements as mutators. This mutagenesis technique was inspired by the syndrome of correlated genetic alterations/defects including high mutation rates that is known as P-M hybrid dysgenesis (ENGELS, 1983). Hybrid dysgenesis results when Drosophila males obtained from natural populations (P strains) are mated with laboratory-reared (M strains) females (ENGELS, 1983). P strains contain numerous copies of the genetic element P, dispersed among their chromosomes. In addition, P strains are characterized by a restrictive cellular environment known as P cytotype that is capable of repressing P element

transposition (BINGHAM, KIDWELL and RUBIN, 1982; RUBIN, KIDWELL and BINGHAM, 1982). Pure M strain chromosomes are devoid of P elements and these strains possess a permissive cellular environment termed M cytotype (ENGELS, 1983). P elements become activated through dysgenic crosses (P strain males X M strain females) which place P element-containing chromosomes in the permissive cellular environment (M cytotype) of the M strain egg (ENGELS, 1983; SIMMONS and BUCHOLZ, 1985). The active state is characterized by transposition events in the hybrid germline that include insertions, excisions, deletions and various chromosome rearrangements mediated by the mobilized P elements. The newly-induced germline mutations are transmitted to the progeny of the dysgenic flies. The insertion of a P element into a new chromosome location can perturb or abolish the function of a gene and in the process give rise to a mutant product (LEVIS, O'HARE and RUBIN, 1984).

The transposition of P elements can occur by either a "replicative" or "conservative" mode (SIMMONS and KARESS, 1985). In the "replicative" mode which involves the transfer of sequence information, a replica of the resident element is made and inserted at a new site while the template element is retained at its original chromosome site. The alternative "conservative" mode would involve transferring the P element to a new chromosome location with

the loss of the original element from its chromosome site. The total number of endogenous elements should not increase as a result of transposition events that are operating under conservative regulatory mechanisms. P elements have been demonstrated to rapidly spread through M cytotype populations supporting the replicative theory of transposition (KIDWELL, NOVY and FEELEY, 1981; KIDWELL and KIYASU, 1984).

P elements can exist in two general forms, complete or intact elements and incomplete or defective elements. The intact P element is 2907 base pairs (bp) with 31 bp terminal inverted repeats flanking the element (O'HARE and RUBIN, 1983). There are four open reading frames and collectively they form a protein product known as "transposase" that is required in trans to catalyze transposition events (KARESS and RUBIN, 1984; LASKI, RIO and RUBIN, 1986; RIO, LASKI and RUBIN, 1986). Defective elements usually contain variable internal deletions of the complete element but retain the terminal inverted repeats. These deletions prevent the element from making a functional transposase product but the element is still capable of transposing if supplied with functional transposase from another source (O'HARE and RUBIN, 1983; RUBIN and SPRADLING, 1982; SPRADLING and RUBIN, 1982). Pseudo-M strains exist which contain only defective P elements among their chromosomes. These pseudo-M strains

are more refractory to transposition events under dysgenic conditions (KIDWELL, 1985). These observations support the theory that defective elements are capable of making a product that regulates the transposition of intact elements (ENGELS, 1986). A positive feedback regulator model originally proposed by O'HARE and RUBIN (1983) suggested that P elements are capable of supplying regulator molecules which suppress transposition and stimulate their own activity. In accordance with this type of model, the permissive cellular environment or M cytotype must lack functional regulator molecules, whereas P cytotype should contain functional regulator molecules.

In assessing whether P elements serve as effective mutators, a number of different P element features must be considered. P element insertions have some site specificity that may be regulated at the nucleotide level or some higher order of chromatin structure (O'HARE and RUBIN, 1983). Various researchers have reported "hotspots" (SIMMONS, et al. 1984) or "coldspots" (SIMMONS and LIM, 1980) for P element insertions under dysgenic conditions, suggesting that this type of mutagenesis would not be feasible for saturation experiments. Some obvious advantages of P element mutagenesis are that transposition is easily controlled with an appropriate mating regime, and newly-induced mutations can be stabilized by maintaining them in a strong P cytotype (ENGELS, 1983). The use of

dysgenically-induced mutations facilitates the cloning of these genes by a technique known as "transposon tagging" (BINGHAM, LEVIS and RUBIN, 1981; BINGHAM, KIDWELL and RUBIN, 1982). P element-induced mutations are usually stabilized by maintaining the mutation in a P cytotype, thus preventing further transposition events. The stabilization of these mutations also retains a P element "tag" or marker inserted at the gene of interest. P element probes are available (i.e. p π 25.1, O'HARE and RUBIN, 1983) and can be used to probe for homologous, recombinant clones containing the gene of interest from cloned libraries made using mutant stock DNA.

X-chromosome lethal mutations induced by P element transposition in males can occur at rates as high as 2.5% (ZUSMAN, 1985). As previously mentioned, dysgenic conditions cause a variety of different mutational events and this can be applied to studies concerned with examining how variability in the expression of a gene/product affects the mutant phenotype. In addition, P element-induced mutations can be reverted under dysgenic conditions at very high frequencies (i.e. 2.6-6.5%, VOELKER et al. 1984), with occasional imprecise excisions that create small deletions in the flanking DNA (DANIELS et al. 1985; VOELKER et al. 1984). All of the aforementioned features need to be considered when interpreting the characteristics exhibited by mutants induced by P elements.

The Cellular Defense System of Drosophila

Drosophila possess an "open type" circulatory system containing a single tubular dorsal vessel which spans the larvae from the third thoracic segment through to the last abdominal segment (RIZKI, 1978). The dorsal vessel is divided anteriorly into the aorta (anterior to the brain-ring gland complex), which lacks ostia, and posteriorly into the heart (abdominal segments 4-5 through 8) (RIZKI, 1978). The lymph glands are the haematopoietic organs of Drosophila and contain three precursor cell types: the prohaemocytes, the proplasmotocytes and the procrystal cells (GATEFF, 1984). The prohaemocytes differentiate into proplasmotocytes which give rise to the group of haemocytes that is normally released into the haemolymph late in the third instar period (GATEFF, 1984). The cellular defense responses in Drosophila are mediated by the haemocytes, which can distinguish between "self" and "non-self" in their phagocytic and encapsulation responses (CHADWICK, 1975; NAPPI, 1975, 1977). Encapsulation is defined as the formation by haemocytes of a cellular envelope around foreign objects (GOTZ, 1986). The three different reactions classified as encapsulation responses are: 1) nodule formation, involving haemocytes and small

particulate material, 2) capsule formation (haemocytic encapsulation), involving haemocytes and foreign objects that exceed the size of haemocytes and 3) humoral encapsulation, involving the formation of melanotic crusts without the participation of haemocytes (GOTZ, 1986). Haemocytes are not involved in the transport of gases to and from internal tissues and are analagous to mammalian leukocytes (NAPPI, 1975). A brief description of the terms used to distinguish morphologically or functionally between the haemocytes of Drosophila is presented in Table 1 (RIZKI, 1978; NAPPI, 1975, 1977).

The ability of an insect to respond when invaded by foreign material requires that active immune-surveillance (or -recognition) mechanisms must be operating in the insect haemocoel. These mechanisms are capable of triggering both cellular and humoral responses but the controlling elements and many of the details remain unresolved. The development of melanotic tumors typically begins with the process of cellular encapsulation. The encapsulation of foreign material that is too large to be phagocytosed involves the aggregation, adhesion and flattening of one haemocyte type (lamellocyte) in a coordinated manner (NAPPI, 1975). Haemocytic encapsulation has also been noted to be stimulated by large masses of particulate matter (RIZKI, 1978). The rapid transformation of rounded haemocytes (plasmatocytes) into long, spindle-

TABLE 1

Drosophila Haemocytes

<p>A. <u>Plasmatocytes and variant forms:</u> (podocyte and lamellocyte)</p>
<ul style="list-style-type: none"> -constitutes the main bulk of haemocyte population -phagocytic cells with adhesive cell surface properties -7 to 12 micron diameter -displays mitotic activity <p style="text-align: center;"><u>Podocytes</u></p> <ul style="list-style-type: none"> -morphological variant of the plasmatocyte -has filamentous or membranous extensions of the cytoplasmic surface -a massive plasmatocyte-podocyte transformation usually occurs during late larval life -may represent the transient intermediate in the differentiation of plasmatocytes to lamellocytes <p style="text-align: center;"><u>Lamellocytes</u></p> <ul style="list-style-type: none"> -flattened, disc-like cells that form the walls of immune capsules -30 to 60 micron diameter; thickness: 0.2 micron -reported to initially appear at 67 h of development (late third instar)
<p>B. <u>Crystal Cells:</u></p>
<ul style="list-style-type: none"> -large, non-phagocytic cells containing non-membrane bound paracrystalline inclusions -cell cytoplasm contains many electron-dense, amorphous bodies -represent 5-10% of larval haemocyte population (all stages) -sole source of haemolymph phenoloxidasases -paracrystalline structure contains both phenoloxidase and tyrosine phenolic substrates -comparable to coagulocytes, spherule cells, oenocytoids and cystocytes of other invertebrates

shaped lamellocytes and the lysis of other haemocytes (crystal cells) occurs during capsule formation (RIZKI, 1978). Flattened lamellocytes surround the foreign material and continue to enwrap the structure until a multi-layered capsule is formed (RIZKI and RIZKI, 1986). Capsule formation is then followed by the intra- and extra-cellular deposition of the pigment melanin late in the third instar stage of development. In some dipterans, melanotic capsules are formed around foreign material without the participation of haemocytes (i.e. haemolymph lacking haemocytes will melanize) (GOTZ, 1986; JONES, 1975; NAPPI, 1975; RIZKI, RIZKI and BELLOTI, 1985; VEY and GOTZ, 1986). This humoral encapsulation event (also called melanosis) usually coincides with low haemocyte counts and generates "protective" polyphenol-protein complexes (NAPPI and CARTON, 1986).

Melanins are formed by the oxidation and polymerization of phenols such as tyrosine and dopa using phenoloxidase enzymes (NAPPI, 1975). Tyrosine and phenoloxidase have both been localized to the paracrystalline inclusion bodies of the crystal cell (NAPPI, 1975; RIZKI, RIZKI and GRELL, 1980; RIZKI, RIZKI and BELLOTI, 1985). The mechanism(s) that regulates crystal cell deposition of melanin is unclear, but there is evidence that supports the involvement of ecdysteroid hormones (RIZKI, 1978; NAPPI, 1977). It has been demonstrated using the *Drosophila*

mutant Black cells (Bc) that amelanotic immune capsules can be formed (RIZKI and RIZKI, 1986). This supports a multi-step encapsulation process in which haemocytic capsule formation is clearly separated from the reactions that form and deposit melanin. In addition, the activation of the precursor prophenoloxidase into its biologically-active form leads to the generation of melanin, parasite destruction, coagulation and opsonization in some invertebrates (GOTZ and VEY, 1986; ROWLEY et al. 1986; SODERHALL and SMITH, 1986; VEY and GOTZ, 1986). This may be due to the exocytotic release of crystal cell components that trigger other humoral and cellular responses (ROWLEY et al. 1986). Although invertebrates lack immunoglobulins and complement they are capable of recognizing and responding to foreignness through an integrated system of agglutinin-like molecules on haemocyte surfaces and humoral factors generated by the prophenoloxidase cascade.

Melanotic Tumor Mutants of Drosophila

The presence of melanotic tumors in Drosophila has been described by many investigators (NAPPI, 1977; GATEFF, 1978a; RIZKI, 1978; SPARROW, 1978; SILVERS and HANRATTY, 1984). Although some of the mutants have been commonly studied by these investigators, there seems to be little

consensus as to the underlying cause(s) of the tumorous phenotype. There is general agreement about the involvement of haemocytes in the encapsulation event, which is then followed by melanin deposition. The specific details, of these two processes in each of the different tumorous mutants are difficult to discern due to significant investigator bias. Melanotic tumors are observed as single or multiple masses either free-floating in the haemocoel or attached to internal tissues (SPARROW, 1978). These melanotic masses appear only infrequently in wild type strains but are consistently found in the tumorous strains. The melanotic tumors generally appear during the third larval instar period in the tumorous strains, and some strains have tumors which persist to the adult stage (SPARROW, 1978).

The melanotic tumor phenotype is usually ascribed to a recessive mutation in one of a number of major genes which, when mutated, give the tumorous phenotype (SPARROW, 1978). There are many minor modifying genes which have the ability to alter the frequency of tumors (SPARROW, 1978). Incomplete penetrance and variable expressivity are phenotypic features associated with the known melanotic tumor mutants, presumably due to the modifier gene influence of the background genotype (SPARROW, 1978).

Studies using the melanotic tumor mutants of *Drosophila* have revealed two general classes of mutants based on the

type of abnormality that initiates the defense response. These two classes are represented by: (Class 1) mutants in which abnormal tissue or cell surfaces trigger a specific autoimmune response and (Class 2) mutants with invasive neoplasms that non-specifically encapsulate tissues or cells. One mutant tu-W, has been extensively studied by Rizki and co-workers and is a typical example of Class 1 melanotic tumor mutants (RIZKI and RIZKI, 1974; RIZKI, 1957; RIZKI, 1978; RIZKI and RIZKI, 1979). Melanotic tumors in tu-W are formed in response to abnormalities that develop in the basement membrane of caudal adipose cells (RIZKI and RIZKI, 1986). It was hypothesized that the degeneration of the basement membrane exposes (or creates) molecules which trigger autoimmune responses (RIZKI and RIZKI, 1986).

In the tumorous-lethal (Tum¹) mutant, neoplastic lymph glands (haematopoietic organs) give rise to an overproduction of precociously-transformed lamellocytes which non-specifically encapsulate "self" tissues (HANRATTY and RYERSE, 1981; NAPPI, KMIECIK and SILVERS, 1984; SILVERS and HANRATTY, 1984). The Tum¹ mutation exhibits a dominant precocious-transformation of haemocytes and a temperature-sensitive, recessive lethality and dominant enhancement of tumorigenesis (HANRATTY and RYERSE, 1981; NAPPI and CARTON, 1986; NAPPI, KMIECIK and SILVERS, 1984; SILVERS and HANRATTY, 1984). The Tum¹ mutation is representative of

the Class 2 type of tumor mutant, in which neoplastic blood-forming organs cause an overproduction of haemocytes that non-specifically encapsulate "self" tissue. It was also demonstrated in this mutant that during infection by live parasites the differentiation of plasmatocytes to lamellocytes was inhibited and the host became more susceptible to the lethal effects of parasitization (NAPPI and CARTON, 1986; NAPPI, KMIECIK and SILVERS, 1984). The inhibition was presumably due to the release of an immunosuppressive agent that prevented encapsulation and melanization events from occurring in the parasitized host. This demonstrated the important role of cellular encapsulation reactions in the prevention of successful parasitic infections in Tum¹ larvae.

The tu-Sz^{ts} mutant also exhibits a dominant precocious-transformation of plasmatocytes to lamellocytes and a temperature-sensitive enhancement of melanotic tumor formation (RIZKI and RIZKI, 1980a; RIZKI and RIZKI, 1980; RIZKI and RIZKI, 1986). At the restrictive temperature (27°C), the encapsulation responses are specific for the abnormal caudal fat body cells of the mutant larvae (RIZKI and RIZKI, 1986). Thus in this mutant, abnormal cellular components are responsible for initiating the reactions leading to melanotic tumor formation. A partial list of some characteristics of the known X-linked melanotic tumor mutants is presented in Table 2.

TABLE 2

X-Linked, Melanotic Tumor or Immune-Defective Mutants

Map Position--Mutant Name (Symbolic name, synonyms)
Phenotype; References

- 0.3 / 1F1; 2A2--deep orange-lethal (dor^1 , 1(1)7)
-males lethal at late 3rd to early pupal stage, abnormal gut, melanotic tumors most prevalent at 25°C, abnormal melanotic anal organ, haemocytes aggregate and melanize in caudal region.^{a,b}
- 1.0 / 3A3--lethal(1)melanomalike (1(1)ml)
-3rd instar lethal, internal melanotic masses.^c
- 20.4--small tumoroid (stu)
-viable, small adult, variable small pseudotumors.^c
- 21.3--lethal(1)8 (1(1)8)
-3rd instar lethal, extended 3rd instar (10 days), testes and lymph glands degenerate, imaginal discs maintain developmental capabilities, abnormal protein metabolism, high free amino acids and peptides.^c
- 29.0--focal melanosis (me)
-late pupal to early adult lethal, tibia/femur junction becomes melanized.^c
- 29.0--pigmy (pig)
-viable, small melanotic adult.^c
- 34.3 / 10A10-11--tumor of Suzuki^{ts} (tu(1)Sz^{ts})
-ts @ 26°C (tumors), melanotic tumors occur in heterozygous females at 26°C, precocious lamellocytes at both permissive and restrictive temperatures (dominant), abnormal caudal fat body.^d
- 34.5--Tumorous-lethal (Tum¹)
-3rd instar to early pupal temperature-sensitive, lethal, hypertrophy/hyperplasia of lymph glands, lymph glands detach from dorsal vessel, gastric caeca absent or reduced, internal melanotic masses, temperature-sensitive at 29°C, overproduction of circulating haemocytes at both permissive and restrictive temperatures.^{e,f}
- 39.0--lethal(1)malignant blood neoplasm (1(1)mbn)
-invasive, transplantable neoplasm, enlarged lymph glands, extensive melanization of larvae including caudal fat body and gut, precocious plasmatocyte-lamellocyte transformations occur.^{a,g}

TABLE 2:
(continued)

41.0--tumor-53 (tu-53)

-viable, low frequency of small melanotic tumors in adults, abnormal/delayed egg hatching, blistered/nicked wings in adults, knotted or shortened wing veins.^c

58.7--tumorous (tms)

-viable, adults slightly small and some with diffuse tumors.^c

64.1--melanized (mel)

-viable, dark adult body color, curled wing tips, dull red eye color.^c

64.5--tumorous head in chromosome 1 (tuh-1)

-weakly viable, has some maternal effect, asymmetrical growths in head region, asymmetrical eye-antennal disc observed in 32 hr. larvae.^c

lethal(1)malignant (l(1)m)--not localized.

-late 3rd to early pupal lethal, lymph gland cells destroy imaginal buds, posterior fat body and testes, tumor cells melanize after tissue damage has occurred.^{a,c}

lethal(1)no differentiation (l(1)nd)--not localized.

-prepupal to pupal lethal, imaginal buds fail to differentiate, imaginal disc mesoderm proliferates abnormally.^c

lethal(1)no imaginal buds (l(1)nib)--not localized.

-3rd instar lethal, small or absent imaginal buds, stomach epithelium excessively proliferates eventually occluding the gut, gut proliferations melanize.^c

a = SPARROW (1978), b = RIZKI (1978), c = LINDSLEY and GRELL (1968), d = RIZKI and RIZKI (1980), e = NAPPI, KMIETIK and SILVERS (1984), f = HANRATTY and RYERSE (1981), g = GATEFF (1978b).

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OBJECTIVES

At the start of this project, a large, P element-mediated mutagenesis experiment was ongoing whose purpose was to isolate new mutations causing altered growth patterns. Approximately 500-1000, X-linked hybrid dysgenesis-induced lethals had been isolated and classified according to their lethal stage. I selected 65 mutations that caused lethality during the late third instar or early pupal stage of development. A more extensive analysis of the lethal syndrome associated with each of these 65 mutants revealed 15 mutants with a heritable melanotic tumor phenotype.

The melanotic tumor mutants were chosen for study because 1) they had an interesting phenotype that was not documented very well in the literature and 2) lymph gland neoplasia had been demonstrated in one X-linked, lethal melanotic tumor mutant. It was expected that these studies would generate information to aid our understanding of the genetic basis of melanotic tumor formation in Drosophila melanogaster. In addition, we hoped that this investigation would determine whether any aberrant growth was caused by the air mutations.

T. K. Johnson, K. R. Hummels and M. Meili found additional dysgenesis-induced mutations with a melanotic tumor phenotype and some of these were included in the

air collection. A. L. Johnson has mapped five additional dysgenesisinduced lethals that exhibited either a melanotic tumor or hypertrophied lymph gland phenotype. These mutants were not allelic to any of the air mutants characterized here, suggesting the existence of additional unidentified X-linked melanotic loci.

INTRODUCTION

The formation of melanotic tumors in Drosophila larvae has been thought to arise as a normal, heritable response to some form of abnormal development (SPARROW, 1978). In third instar larvae of melanotic tumor strains, cellular or haemocytic capsules are formed around aberrant larval tissue, and then melanin is deposited within and around the capsule (RIZKI and RIZKI, 1986). This type of cellular defense response also will occur normally if foreign objects invade the larval haemocoel, such as during parasitization or wounding events (GOTZ, 1986). The melanotic tumor phenotype typically shows a recessive mode of inheritance with incomplete penetrance and variable expressivity, apparently due to numerous modifier genes present in the genome (SPARROW, 1978).

We propose that melanotic tumor mutants can be divided into two general classes based on the type of abnormality which initiates the defense response. These two classes are represented by: (1) mutants in which abnormal tissue or cell surfaces trigger a specific autoimmune response (RIZKI and RIZKI, 1974a; RIZKI and RIZKI, 1980a) and (2) mutants with invasive neoplasms that non-specifically encapsulate tissues/cells (ie. lymph gland neoplasia, HANRATTY and RYERSE, 1981). Although a large number of non-allelic loci can be mutated to give a melanotic tumor phenotype, the

genetic basis of immune recognition and the regulation of the defense responses are still poorly understood. In a P elementmediated mutagenesis experiment, we have isolated 20 lethal mutations exhibiting a melanotic tumor phenotype. Experiments were conducted to determine the number of distinct X-chromosome loci represented in this collection of mutants. The lethal syndromes were characterized in an effort to identify specific developmental anomalies that might be associated with melanotic tumor formation in each mutant.

MATERIALS AND METHODS

Culture Conditions: All fly strains were maintained on standard cornmeal, molasses, yeast and agar Drosophila medium in vials or half-pint milk bottles. The X-chromosome duplications and deficiencies used in this study are listed in Table 3. Descriptions of other mutations and chromosomes can be found in LINDSLEY and GRELL (1968) and LINDSLEY and ZIMM (1986, 1987). Fly stocks were obtained from the following sources: California Institute of Technology Stock Center; Mid-America Drosophila Stock Center, Bowling Green State University; Drs. P. J. Bryant, A. T. C. Carpenter, W. R. Engels, B. W. Geer, J. L. Marsh, N. Perrimon, C. A. Poodry, T. M. Rizki, M. J. Simmons and D. F. Woods. P strain stocks were constructed by repeatedly backcrossing flies to a strong P strain (π^2).

Mutagenesis Scheme: X-chromosome lethal mutations were induced using P element mobilization under dysgenic conditions as outlined in Figure 1. The isogenized X-chromosome stock used in the mutagenesis was marked with yellow¹, white¹ and a cryptic weak bobbed allele. The dysgenic F1 males were crossed to P-strain females bearing a balanced X-chromosome lethal mutation. Newly-induced recessive lethal mutations were identified by the absence of male progeny bearing the mutagenized chromosome. These lethal bearing chromosomes were recovered by crossing the

TABLE 3
X-Chromosome Duplications / Deficiencies.

Duplication Name	X-Chromosome Breakpoints	Ref.	Deficiency Name	X-Chromosome Breakpoints	Ref.
v ⁺ Yy ⁺	1A1;1B2;Y	1	Df(1)svr	1A1;1B10-13	2
Dp(1;2)Bld	1A1;1C3-4	2	Df(1)A94	1E3;2B15	1
y ² Y67g19.1	1A1;2B17-18	1	Df(1)S39	1E4;2B11-12	1
Dp(1;3)sta	1D3-E1;2A1	2	Df(1)Pg ^d 35	2D3;2E2-F5	1
Dp(1;3)w ^{vco}	2B17-C1;3C6	2	Df(1)64c18	2E1-2;3C2	1
B ^S w ⁺ y ⁺ .Y	2D1;3C7	3	Df(1)TEM304	2E2-F1;3A4-6	2
Dp(1;2)w ^{+70h}	3A7-8;3C2-3	2	Df(1)JC19	2F3;3C5	1
Dp(1;2)w ^{+51b7}	3C1-2;3D6-7	1	Df(1)w ²⁵⁸⁻⁴²	3A4-6;3C5-6	2
Dp(1;2)w ^{+64b13}	3C2;5A1-2	1	Df(1)w ²⁵⁸⁻⁴⁸	3A9-B1;3C2-3	2
Dp(1;2)rb ^{+71g}	3F3;5E8	1	Df(1)HF366	3E7-8;5A7	2
Dp(1;3)sn ^{13a1}	6C11;7C9	1	Df(1)C149	5A8-9;5C5-6	1
y ⁺ ct ⁺ y	6E;7C4-6	3	Df(1)N73	5C2;5D5-6	1
Dp(1;2)sn ^{+72d}	7A8;8A5	1	Df(1)HA32	6E4-5;7A6	1
Dp(1;2)v ^{+75d}	9A2;10C2	1	Df(1)ct ⁷⁸	6F1-2;7C1-2	2
Dp(1;2)v ⁺⁶³ⁱ	9E1;10A11	1	Df(1)ct ^{J4}	7A2;7C1	1
v ⁺ Yy ⁺	9F3;10C1-2	1	Df(1)ct ^{4b1}	7B2;7C4	2
v ⁺ Yy ⁺³	9F3;10E3-4	1	Df(1)C128	7D1;7D5-6	2
Dp(1;2)v ^{65b}	10A1;11A7	1	Df(1)RA2	7D10;8A4-5	1
Dp(1;4)r ⁺ f ⁺	13F10;16A1-2	2	Df(1)KA14	7F1-2;8C6	2
Dp(1;2)r ^{+75c}	14B13;15A9	1	Df(1)v ^{L15}	9B1;10A1	1
Dp(1;3)f ^{+71b}	15A4;16C2-3	1	Df(1)HC133	9B9-10;9E-F	2
			Df(1)v ^{L3}	9F6-9;10A6-7	1
			Df(1)v ^{L2}	9F13;10A1	2
			Df(1)RA37	10A6-7;10B15-17	2
			Df(1)KA7	10A9;10F10	1
			Df(1)N71	10B5;10D4	1
			Df(1)DA622	10B6-8;10D2	2
			Df(1)M13	10B10;11A3-7	5
			Df(1)N105	10F7;11D1	2
			Df(1)N12	11D1-2;11F1-2	2
			Df(1)sd ^{72b26}	13F1;14B1	2
			Df(1)D15	14D1;15C5	4
			Df(1)D17	14F6;15A6	2

1. CRAYMER and ROY (1980).
2. LINDSLEY and ZIMM (1987).
3. JOHNSON and JUDD (1979).
4. T. K. JOHNSON (PERS. COMM.).
5. D. F. WOODS (PERS. COMM.).

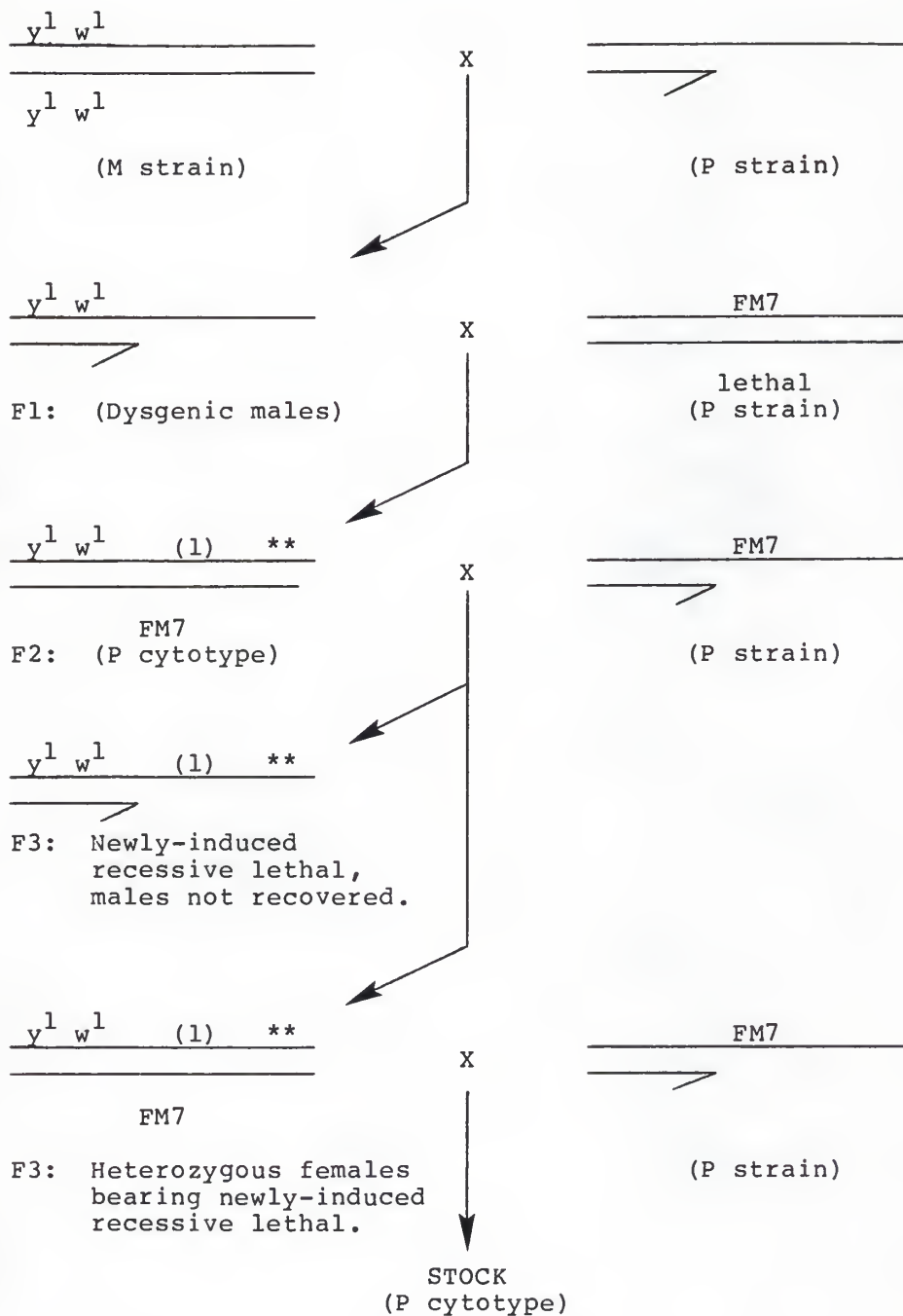


Figure 1.-Mutagenesis Scheme. ** is the mutagenized chromosome.

lethal/balancer heterozygous sibs to P strain males.

Assessment of the Melanotic Tumor Phenotype: A group of newlyinduced, sex-linked mutations with a terminal lethal phase during the late third instar or early pupal stage were assessed to determine whether they caused heritable "melanotic pseudotumors" (referred to as melanotic tumors in this article). The hemizygous, mutant larvae from each lethal stock could be identified due to the existence of a y^1 marker on the mutagenized chromosome. These mutant males were examined for melanotic or brown/black masses in the haemocoel of late third instar larvae. The mutations described in this study which give rise to melanotic tumors as a result of a genetic lesion have been designated aberrant immune response mutations or air. Other lethal point mutant and deficiency stocks also were examined for the presence of melanotic tumors during the late third instar period of development.

Genetic Mapping Protocols: Approximate map positions for the air mutations were determined initially by recombination analyses using the following multiply marked X-chromosomes: sc ec cv ct v g f, y pn cv m f bb^{2rl3} and sc ec cv ct m v f (see Appendices 1, 2, 3 and 4). The duplications and deficiencies listed in Table 3 were employed where applicable, to cytologically localize the air mutations along the X-chromosome. Mutant air males that had been rescued from lethality were obtained by

crossing $y^1 w^1$ air/balancer heterozygous females with males bearing an appropriate X-chromosome duplication (see Appendix 5). These duplication-covered male air offspring were identified by the presence of the $y^1 w^1$ markers in adult males. The air mutations were determined to be within the confines of a deficiency when air/deficiency heterozygotes were not recovered in the progeny of a cross.

Lethal Complementation Analyses: In some cases, the air lethals were tested for their ability to complement functionally, other mutations which had been localized to a common chromosome subregion. Duplication-covered air males were crossed to females heterozygous for other air mutations or lethal point mutations previously isolated by others, and the progeny were scored for the presence of lethal/air or air/air females in the absence of the duplication (see Appendix 6). The inability to recover the lethal/air or air/air combination in females lacking the duplication was the criterion used to assign allelism.

Analysis of Lethal Phase: Hemizygous air larvae were followed from hatching to determine the stage at which development was arrested. Heterozygous $y^1 w^1$ air/Binsn (P cytotype) females from each air stock were allowed to mate with Binsn (P cytotype) males for 4 days at 25°C, after which serial egg collections were made using replaceable petri plates filled with standard Drosophila medium. The total number of eggs on each plate was determined and the

percent of larvae hatching was calculated using the formula: (total number of larvae/total number of eggs X 100). Male air larvae had light brown mouthparts due to the y^1 marker on the air chromosome and were easily distinguished from larvae of other genotypes, which had dark brown or black mouthparts (see Appendix 7). The air male larvae were sorted from the other larvae and placed in separate vials to follow their development. Larvae were scored for the most terminal stage of development reached; for simplicity these stages were divided as follows: Early Larvae (EL) = 1st and 2nd instar larvae, Late Larvae (LL) = 3rd instar larvae, Pseudopupae (Psp) = larvae with some pupal characteristics, Early Pupae (EP) and Adults (A) = revertants. Non-mutant larvae also were transferred to vials and followed until eclosion to determine whether the experimental manipulation had affected their survival rate. Mortality in these controls was minimal and it was assumed that the larvae were not adversely affected by the treatment. Lethal periods during the embryonic period of development were tentatively assigned when there were departures from the expected 25% genotypic ratio of air males in the progeny larvae.

Developmental Characteristics of air Larvae: Male larvae from each air stock were subjected to a preliminary characterization of their internal morphology. Larvae were dissected in Ringer's solution and examined with the aid of

a dissecting microscope. This analysis included a crude morphological examination of the anterior midgut, brain hemispheres, fat body, gastric caeca, imaginal discs, lymph glands, Malpighian tubules, proventriculus, ring gland, salivary gland and ventral ganglion. Since the hemizygous male larvae from all air stocks develop more slowly than do their sibs or wild type counterparts, the stage of development that preceded their most terminal stage was chosen for dissection. Larval characteristics were reported only when all morphological features could be scored on one individual.

Reversion Mutagenesis: Dysgenic hybrids were generated for a few selected air stocks to analyze the frequency of reversion of the lethal phenotype when placed under dysgenic conditions. This provided an indirect assay that P element inserts were responsible for the air mutant phenotype in these stocks. Duplication-covered air males (P strain) were crossed to attached-X females (M strain) bearing the same duplication. Individual F1 dysgenic males then were crossed to attached-X females that lacked the duplication. The total number of F2, attached-X females and any $y^1 w^1$ revertant adult males were scored. The reversion frequency was calculated using the formula: (revertant males + attached-X females X 100).

RESULTS

In this study, 20 recessive P element-induced lethal mutations giving rise to melanotic tumor phenotypes were identified and some of their properties were studied. This lethal, melanotic tumor phenotype was observed in approximately 1.2% of all the X-linked lethal mutations generated in our mutagenesis experiment. The characteristic air mutant larval phenotype is shown in Figure 2.

Genetic Mapping: Initially, it was important to determine the number of distinct X-chromosome loci that were represented by the air mutants. Each air mutation was mapped by recombination to provide an approximate map position and also to generate males that were recombinant for the chromosome interval surrounding the air location. Lethality was the phenotypic marker used in all of the recombination mapping experiments, for the melanotic tumor phenotype in every air mutant was characterized by incomplete penetrance and variable expressivity. The lethal and melanotic tumor aspects of each air mutant phenotype were tested to determine whether they were pleiotropic effects of a single gene mutation or two linked mutations. All males recombinant for the marker interval containing the lethal locus were crossed to attached-X females and the stocks were maintained for many

FIGURE 2.- Melanotic tumor phenotype of selected air mutants. A, air1. B, air6. C, D, air8. E, air10. F, air13.

A



air1

B



air6

C



air8

D



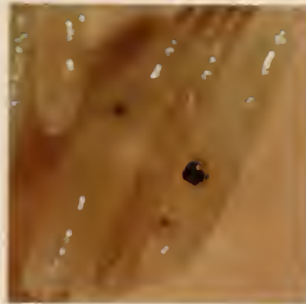
air8

E



air10

F



air13

generations. The male offspring from these crosses were examined during late larval stages for any evidence of melanotic tumors, which would indicate that a putative mutation causing this phenotype had been separated from the lethal. The frequency of melanotic tumors observed for any of the recombinant males generated from the air mutant recombination crosses was never greater than that observed in the original M strain (y¹ w¹ stock) used in the mutagenesis (data not shown). Light brown pigmentation was observed at the anterior portion of the fore-/hind-gut junction in the original y¹ w¹ stock and in most of the recombinant male stocks. It should be noted that this pigmentation was distinctly different in appearance from the pigmentation associated with the air melanotic tumor phenotype.

A more precise map position for each air mutation was obtained through the use of numerous X-chromosome duplications and deficiencies (Table 3). A cytogenetic map showing the distribution of duplications, deficiencies and air mutant positions in four subsections of the X chromosome is presented in Figure 3. The cytological locations of airl1, airl3, airl4 and airl5 are not displayed in Figure 3, because these mutations could not be rescued from lethality using any of the duplications listed in Table 3. The mutation labelled airl6^d was recovered rarely in males bearing the duplication segregant of

FIGURE 3.- Cytogenetic map of the polytene X-chromosome. The thin solid bars show the cytological extent of various duplications and the dashed bars represent the limits of X-chromosome deficiencies. The genetically-determined map positions of the air mutants based on coverage and allelism tests are indicated by thick solid bars. A, distal region of the X-chromosome including bands 1-4. B, polytene band interval 5-7. C, polytene band interval 9-11. D, polytene band interval 13-17.

A

TEM304 w²⁵⁸⁻⁴⁸

JC19

64C18

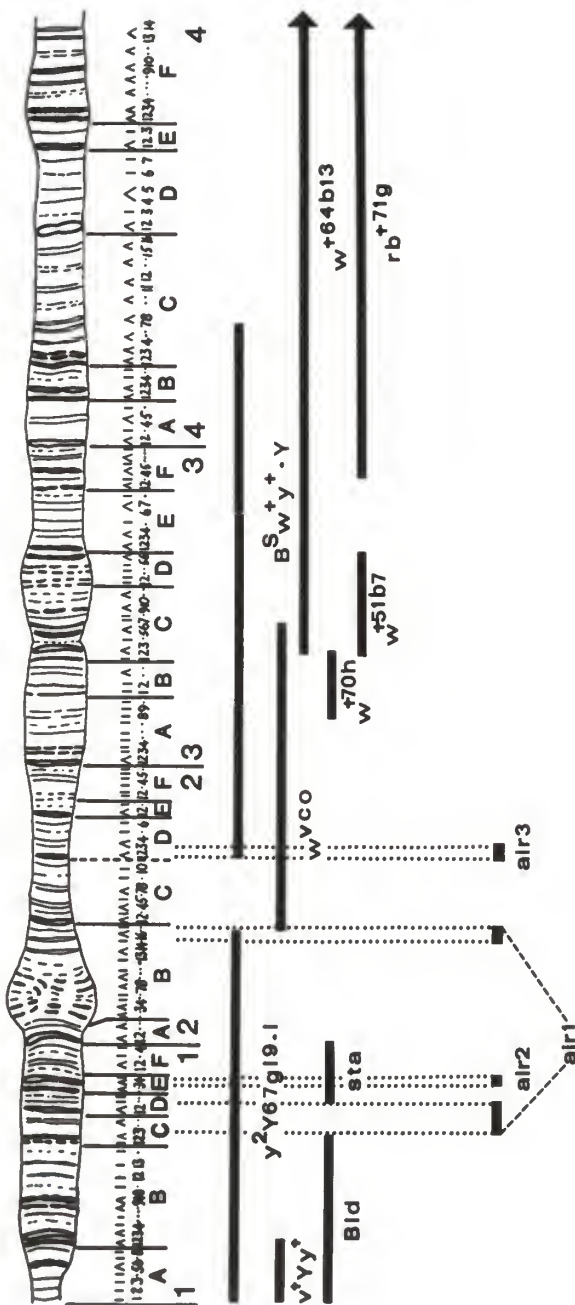
Pgd³⁵ w²⁵⁸⁻⁴²

HF366

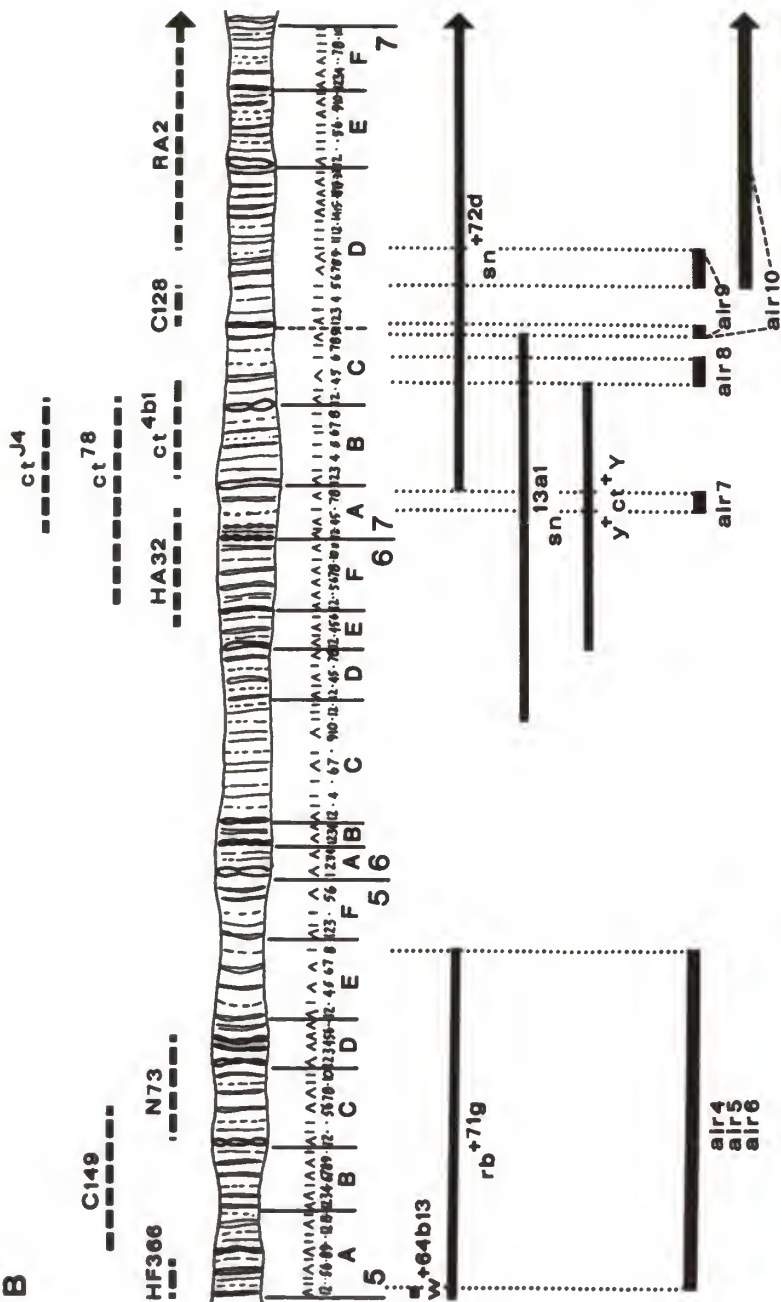
S39

A94

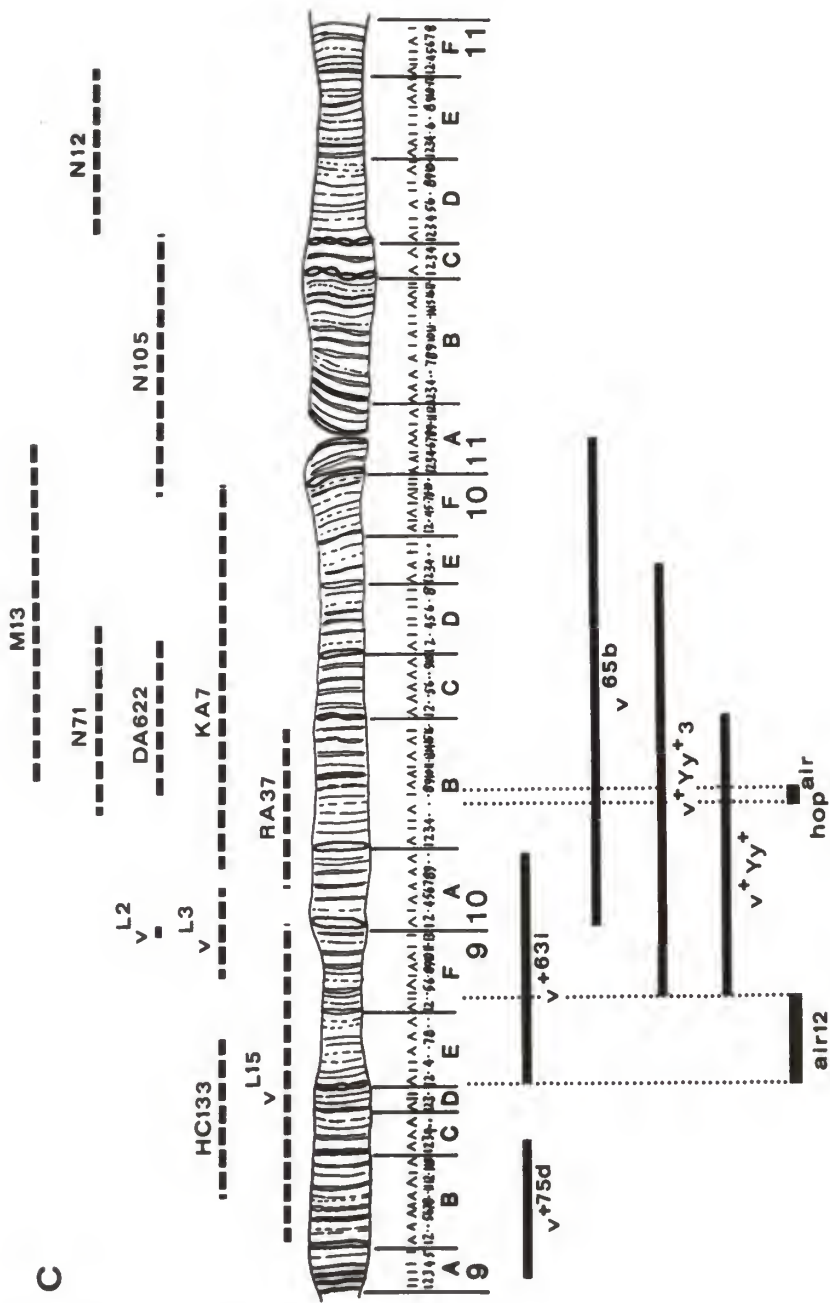
svr



B



C



D

D17

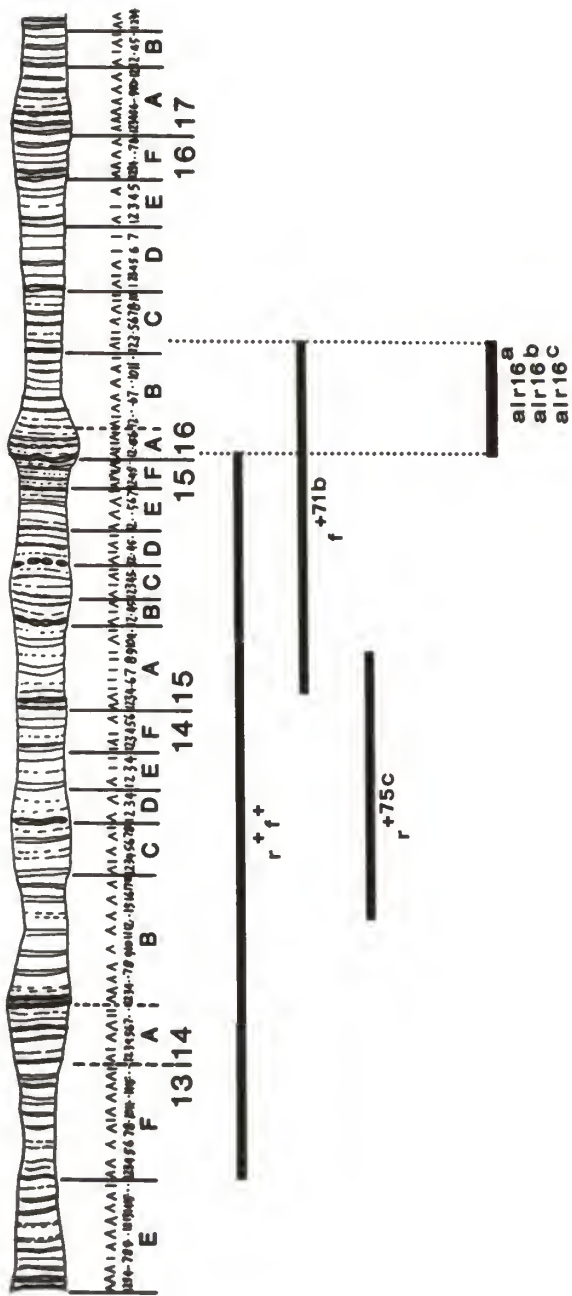
D15

72b26

sd

+75C

r



T(1;3)f^{+71b}, but all of these males were infertile. The recombination map positions of the mutants: air11, air13, air14, air15 and air16^d are listed in Table 4, along with their tentative cytological locations derived collectively from recombination, duplication and deficiency mapping (see Appendix 9). The cytological location of the other air mutants also are given in Table 4.

Lethal Complementation Results: Inter se crosses were performed among those air mutants that had been localized to coincident chromosome regions and where duplication-covered, fertile males were available. All heterozygous combinations of the four independently isolated air16 mutations failed to complement each other and were assigned to a single complementation group. No other cases of allelism between air mutations could be detected. Some air mutants were tested for their ability to functionally complement point mutants previously localized to similar chromosome regions. A compilation of complementation results is given in Table 5. The mutant air7 was determined to be allelic to the point mutants: 1(1)7Ac², 1(1)7Ac³, 1(1)7Ac⁵ and 1(1)7Ac⁶ previously known as 1(1)HF302, 1(1)JA59, 1(1)RC57 and 1(1)RC61 respectively (LINDSLEY and ZIMM, 1986). In repeated tests, 1(1)7Ac⁷ (1(1)EF465) always complemented air7 and the other members of the complementation group namely, 1(1)7Ac², 1(1)7Ac³, 1(1)7Ac⁵ and 1(1)7Ac⁶ (LINDSLEY and ZIMM, 1986). Thus, the

TABLE 4
Summary of Map Positions--air Lethals
Map Positions

<u>Mutant</u>	<u>Recombination</u>	<u>Cytological</u>
<u>air1</u>		1C3-4; 1D3-E1 2B15; 2B17-18
<u>air2</u>		1E3; 1E4
<u>air3</u>		2D1; 2D3
<u>air4</u>		5A1-2; 5E8
<u>air5</u>		5A1-2; 5E8
<u>air6</u>		5A1-2; 5E8
<u>air7</u>		7A6; 7A8
<u>air8</u>		7C4; 7C9
<u>air9</u>		7C9; 7D1 7D5; 7D10
<u>air10</u>		7C9; 7D1 7D5; 8A5
<u>air11</u>	29.0	(8A5; 9A2) (11A7; 13F10)
<u>air12</u>		9E1; 9F3
<u>air13</u>	35.4	(8A5; 9A2) (11A7; 13F10)
<u>hop</u> ^{air}		10B6; 10B8
<u>air14</u>	44.9	(11A7; 13F10)
<u>air15</u>	55.6	(11A7; 13F10)
<u>air16</u> ^a		16A1-2; 16C2-3
<u>air16</u> ^b		16A1-2; 16C2-3
<u>air16</u> ^c		16A1-2; 16C2-3
<u>air16</u> ^d	56.7	(16A1-2; 16C2-3)

() = Approximate cytological locations based on recombination, duplication and deficiency mapping. See Appendix 9.

TABLE 5A

Lethal Complementation Analyses.
(Polytene Band Interval: 1-3).

Mutant (Map Position)	1	2	3	4	5
1. <u>dor</u> ¹ (1F1;2A2)		-	-		
2. <u>air</u> ¹ (1C3-4;1E3 2B15;2B17-18)	-	-			-
3. <u>air</u> ² (1E3;1E4)	-				-
4. <u>air</u> ³ (2D1;2D3)					-
5. <u>l(1)ml</u> (3A3)		-	-	-	

+ = fails to complement.
- = complements.

TABLE 5B

Lethal Complementation Analyses.

(Polytene Band Interval: 7).

The map positions listed for the mutants l(1)X-6 and l(1)26 were based on their inclusion within the limits of the duplication y⁺ct⁺Y (6E;7C4-6) and their exclusion from Df(1)ct^{4b1} (7B2;7C4) (T. K. JOHNSON, PERS. COMM.). The mutant air8 was not rescued by the duplication y⁺ct⁺Y nor was it allelic to Df(1)ct^{4b1}. (+ = fails to complement, - = complements).

Mutant (Map Position)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. $\frac{\text{air7}}{(7A6;7A8)}$		-	+	+	+	+	-	-	-	-	-	-	-	-	-	-
2. $\frac{1(1)25}{(7A6;7A8)}$	-															
3. $\frac{1(1)7Ac^2}{(7A6;7A8)}$	+					+	-									
4. $\frac{1(1)7Ac^3}{(7A6;7A8)}$	+															
5. $\frac{1(1)7Ac^5}{(7A6;7A8)}$	+						-									
6. $\frac{1(1)7Ac^6}{(7A6;7A8)}$	+		+				-									
7. $\frac{1(1)7Ac^7}{(7A6;7A8)}$	-		-		-	-										
8. $\frac{\text{air8}}{(7C4-6;7C9)}$	-								-	-	-	-	-	-	-	-
9. $\frac{1(1)X-6}{(7C4;7C6)}$	-							-			-	-	-	-	-	-
10. $\frac{1(1)26}{(7C4;7C6)}$	-							-								
11. $\frac{1(1)7Cc^2}{(7C6;7D1)}$	-							-	-							
12. $\frac{1(1)7Cc^4}{(7C6;7D1)}$	-							-	-							
13. $\frac{1(1)14}{(7C6;7D1)}$	-							-	-							
14. $\frac{1(1)7Ce^1}{(7C6;7D1)}$	-							-	-							
15. $\frac{1(1)7Cg^1}{(7C6;7D1)}$	-							-	-							
16. $\frac{1(1)7Ch^1}{(7C6;7D1)}$	-							-	-							

TABLE 5C

Lethal Complementation Analyses.

(Polytene Band Interval 9-10).

a = HANRATTY and RYERSE (1981). The following alleles were also tested: b = $\frac{1(1)10Ba^2}{-}$; c = $\frac{1(1)10Bb^{12}}{-}$; d = $\frac{dsh^{M20}}{-}$, $\frac{dsh^{VA153}}{-}$; e = $\frac{hop^{VE666}}{-}$, $\frac{hop^{HC257}}{-}$, $\frac{hop^{L4}}{-}$, $\frac{hop^{VA85}}{-}$, $\frac{hop^{GA32}}{-}$, $\frac{hop^{C111}}{-}$. The map positions for d and e were obtained from PERRIMON and MAHOWALD (1986, 1987). The map position for f was from D. F. WOODS (PERS. COMM.).

(+ = fails to complement, - = complements).

Mutant (Map Position)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. <u>air</u> ¹² (9E1;9F3)			-					-					-	-			
2. <u>1(1)9Fe</u> ¹ (9F9;9F12)								-					-	-			
3. <u>1(1)9Fh</u> ⁴ (9F4;9F8)			-					-					-				
4. <u>1(1)9Fi</u> ² (9F13;10A1)			-					-									
5. <u>1(1)10Ae</u> ¹⁴ (10A6;10A7)			-					-									
6. <u>1(1)10Ag</u> ⁸ (10A9)								-					-				
7. <u>1(1)10Ah</u> ³ (10A8;10A11)								-					-				
8. <u>Tum</u> ¹ (1-34.5) ^a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9. <u>1(1)10Ba</u> ¹ (10B4;10B9) ^b								-					-				
10. <u>1(1)10Bb</u> ⁹ (10B4;10B9) ^c								-									
11. <u>1(1)10Bc</u> ¹ (10B4;10B9)								-					-				
12. <u>dsh</u> ¹ (10B5;10B7) ^d								-					-				
13. <u>hop</u> ^{air} (10B6;10B8)	-	-	-			-	-	-	-	-	-	-		+	-	-	-
14. <u>hop</u> ^{v48} (10B6;10B8) ^e	-	-						-					+				
15. <u>dlg</u> ¹ (10B8;10B9) ^f								-					-				
16. <u>1(1)10Bi</u> ¹ (10B17;10C2)								-					-				
17. <u>1(1)10Bj</u> ¹ (10B17;10C2)								-					-				

presumed 1(1)7Ac⁷ allele did not behave as previously described (Table 5B). The mutant designated hop^{air} was shown to be an allele of the hopscotch locus by its failure to complement hop^{v48}, hop^{VE666}, hop^{HC257}, hop^{L4}, hop^{VA85}, hop^{GA32} and hop^{C111} (Table 5C).

Lethal Phase Analyses: The lethal phase for the air mutants was determined by mating Binsn males (P cytotype) to y¹ w¹ air/Binsn females (P cytotype) and following the development of the hemizygous y¹ w¹ males. The first column in Table 6A gives the percentage of air males that were represented in the total pool of hatched larvae. The degree of embryonic lethality (Emb.) exhibited by the air mutants was calculated by subtracting the percent of air male larvae (derived from heterozygous mothers) from the 25% expected for this class, with the difference representing lethality during the embryonic stage of development. The larval lethal phases were determined by scoring the developmental fate of all Fl, air males that hatched into larvae. The proportion of hatched larvae that died at Early Larval (1st and 2nd instar), Late Larval (3rd instar), Pseudopupal (larval and pupal characteristics), Early Pupal or Adult stages was determined for each air mutant. The Pseudopupal (Psp) classification usually was applied to individuals with shortened larval cuticles, some cuticular hardening and early signs of pigmentation. The early pupal (EP) label was applied equally to individuals

TABLE 6A

Lethal Phases of air Mutations.

Binsn males were crossed to y¹ w¹ air/Binsn females and their F1's were scored. Embryonic lethality (Emb.) was calculated by subtracting the percent of hemizygous (y¹ w¹ air) F1 males hatching from the 25% expected for that progeny class. The larval/adult lethal periods were determined by scoring the stage at which the F1 y¹ w¹ air males arrested development using the following stages:
A =adults (revertants); EL = 1st & 2nd instar larvae; EP = early pupae; LL = 3rd instar larvae; Psp = pseudopupae (larvae with some pupal features).

Mutant	F1: <u>air</u> male larvae (%)		Lethal Phase-- <u>air</u> male larvae(%)					# Eggs Scored
	Hatched Emb.		EL	LL	Psp	EP	A	
<u>air1</u>	25.1	---	42.3	55.9	1.8			512
<u>air2</u>	26.5	---	6.7	3.6	-----	89.1	0.6	740
<u>air3</u>	19.5	5.5	10.3	17.2	36.8	35.6		568
<u>air4</u>	24.6	0.4	38.8	61.2				445
<u>air5</u>	22.4	2.6	18.4	6.6	55.9	19.1		670
<u>air6</u>	25.5	---	33.6	21.7	44.8			1213
<u>air7</u>	24.2	0.8	51.9	17.6	29.6	0.9		594
<u>air8</u>	21.4	3.6	43.6	13.7	38.5	2.6	1.7	660
<u>air9</u>	22.4	2.6	30.8	20.5	48.7			588
<u>air10</u>	24.6	0.4	50.6	35.4	7.6	6.5		1183
<u>air11</u>	21.6	3.4	67.0	14.0	12.0	5.0	2.0	594
<u>air12</u>	25.2	---	5.2		0.9	93.9		949
<u>air13</u>	23.6	1.4	9.7	1.4	89.0			712
<u>hop</u> ^{air}	25.0	---	74.9	2.1	2.6	20.0	0.5	928
<u>air14</u>	27.1	---	39.0	48.0	12.4	0.6		707
<u>air15</u>	24.9	0.1	30.7	7.9	0.5	60.8		828
<u>air16</u> ^a	24.1	0.9	59.5	36.7	2.7	1.1		1250
<u>air16</u> ^b	26.2	---	55.7	19.2	11.4	13.8		768
<u>air16</u> ^c	22.4	2.6	37.0	33.3	13.0	16.7		501
<u>air16</u> ^d	22.4	2.6	78.2	14.9	6.9			519

that formed "normal" puparia and then ceased development and to those individuals that actually pupated. Most of the air mutants were characterized by multiphasic lethality (Table 6A). The percent of larval lethality at different stages was based on the ratio: number of air males that reached each stage/total air male larvae that hatched. The predominant lethal phase for each air mutant is listed in Table 6B.

The developmental progression of each air mutant was examined with respect to two parameters. These parameters were: 1) delayed developmental rates and 2) the inability to undergo metamorphosis as evidenced by an extended period of time at the third instar stage. When the air mutants were examined for evidence of extended late larval periods distinct from their delayed developmental rate, there was no indication of heterogeneity in developmental rate for the mutants air4, air5, air12, air16^a and air16^d. A subset of hemizygous air1, air2, air3, air7, air9, air11 and air16^b males were found as active third instar larvae, 7 days after the eclosion date of their non-mutant sibs. Some third instar male larvae that were genotypically air6, air8, air10, air13 or air15 survived up to 14 days after some mutant sibs had ceased development and their non-mutant sibs had eclosed.

Developmental Characteristics of air Mutants:

Preliminary characterization was made of the larval anatomy

TABLE 6B

Predominant Lethal Period--air Mutants.

Early Larvae (EL)	Late Larvae (LL)	Pseudopupae (Psp)	Early Pupae (EP)
<u>air7</u>	<u>air1</u>	<u>air2</u>	<u>air2</u>
<u>air8</u>	<u>air4</u>	<u>air3</u>	<u>air3</u>
<u>air10</u>	<u>air14</u>	<u>air5</u>	<u>air12</u>
<u>air11</u>		<u>air6</u>	<u>air15</u>
<u>hop</u> ^{air}		<u>air9</u>	
<u>air16</u> ^a		<u>air13</u>	
<u>air16</u> ^b			
<u>air16</u> ^c			
<u>air16</u> ^d			

of each air mutant. The ring gland, lymph glands, salivary glands, brain, gut, and imaginal discs were examined for any morphological defects or anomalies. The primary aim of this analysis was to correlate specific defects or tissue involvement with the melanotic tumor phenotype. This was accomplished by recording all melanized structures and melanotic tumors that were observed during the dissections of the mutant larvae. One defect that was common to all air mutants was a small and/or abnormal brain (Table 7). Hemizygous air2, air6, air7, air8, air9, air11, air13 and air15 males were shown to have enlarged lymph gland lobes when compared to the $y^1 w^1$ (M strain) "wild type" stock. Montages displaying the morphology of the brain complex, ring gland, dorsal vessel and lymph glands of air13 (P strain) and $y^1 w^1$ (M strain) male larvae are shown in Figure 4. The hypertrophied lymph glands of air6 and air11 males are depicted in Figure 5. Melanotic nodules located in the most anterior pair of the lymph gland lobes can be seen in Figure 5A and C. Melanotic nodules also were observed in the hypertrophied lymph glands of the following mutants: air2, air7, air8, air9, air11 and air13 (Table 7). In addition, the lymph glands (not hypertrophied) of air4 and air12 mutant males also contained melanized nodules. An increase in the total number of lymph gland lobes was observed in air13 mutant larvae (Figure 6). Many air13 individuals contained greater than ten

TABLE 7

Developmental Characteristics of the air Mutants

<u>Mutant</u>	<u>Ring Gland</u>	<u>Lymph Glands</u>	<u>Salivary Glands</u>	<u>Brain</u>	<u>Gut</u>	<u>Imaginal Discs</u>	<u>Melanized Structures</u>
<u>air1*</u>	sm	norm-lg	sm	Bh-sm Vg-sm	In-mel	Eye/an-m Wing-v	hind-In
<u>air2</u>	sm-norm ab	lg/mel	norm	Bh-ab Vg-sm	AM -v P -lg	Eye/an-ab Wing-v	Lymph gland
<u>air3</u>	norm/ab	norm	sm-norm	Bh-ab Vg-ab	In -mel	Eye/an-ab	ff hind-In Trachea
<u>air4*</u>	sm	sm-norm mel	sm-norm	Bh-sm Vg-sm	AM -lg Gc -m/sm In -mel P -lg/mel	m/ab	hind-In Lymph gland
<u>air5</u>	sm	norm	sm	Bh-ab/sm Vg-sm	In -mel P -ab	v	ff hind-In
<u>air6</u>	sm-norm	lg	sm-norm	Bh-ab Vg-sm	Gar-ab Gc -ab P -lg/mel	Eye/an-ab sm	Proventriculus
<u>air7</u>	sm	lg/mel	sm	Bh-sm Vg-sm	In -mel/ab P -sm ab	m-sm ab	hind-In Lymph gland Trachea
<u>air8</u>	sm/ab	lg/mel	sm	Bh-ab Vg-ab	AM -lg Gar-ab	ab Wing-v	ff Garland cells

TABLE 7
(continued)

<u>air9*</u>	sm	v/mel	sm-norm	Bh-ab Vg-sm	Gc -mel -sm P -ab	Gonads Lymph gland
<u>air10*</u>	sm	norm-lg	norm	Bh-sm Vg-sm	In-mel	ff AM/hind-In Lymph gland
<u>air11</u>	sm	lg/mel	sm	Bh-ab Vg-sm	Gc -sm P -ab	hind-In Lymph gland
<u>air12</u>	sm-norm	norm/mel	norm	Bh-ab Vg-sm	Gar-mel Gc -v P -ab	hind-In Lymph gland mid-In
<u>air13</u>	sm	v/mel	sm	Bh-ab Vg-sm	Gc -v	ff Lymph gland
<u>hop^{air*}</u>	sm	norm	norm	sm	Gc -lg P -ab	ab Wing-ab -m
<u>air14*</u>	sm/ab	norm	sm-norm	Bh-sm Vg-sm	norm	sm Wing-ab
<u>air15</u>	sm	lg	sm-norm	Bh-sm Vg-sm	Gc -ab P -sm	Trachea
<u>air16^{a*}</u>						sm/ab

TABLE 7
(continued)

<u>air16</u> ^{b*} sm	sm-norm	v	Bh-sm Vg-sm	Gar-mel In -mel	sm-m	ff Garland cells hind-In Trachea
<u>air16</u> ^{c*} sm-norm	norm-lg	norm	Bh-sm	In -mel	m	ff hind-In
<u>air16</u> ^{d*} sm	m	sm	Bh-sm	norm	sm-m	

A minimum of 10 (except *) air male larvae were dissected at late third instar or the most terminal developmental stage reached. Abbreviations are: AM = Anterior midgut, Eye/an = Eye-antennal discs, Gar = Garland cells, Gc = Gastric caeca, Bh = brain hemispheres, In = intestine, P = proventriculus, Vg = Ventral ganglion, ab = abnormal, ff = free-floating in haemocoel, lg = large, m = missing, mel = melanized, norm = normal, sm = small, v = variable.

FIGURE 4.- Montages depicting the brain complex, ring gland, dorsal vessel and lymph gland anatomy of late third instar larvae. A, y¹ w¹ air13 (P strain) mutant exhibiting hypertrophy in the lymph glands and B, y¹ w¹ (M strain) larva with wild type lymph gland morphology. Abbreviations are: BH, brain hemispheres; DV, dorsal vessel; ID, imaginal discs, LG, lymph glands; PC, pericardial cells; RG, ring gland; VG, ventral ganglion. Magnification A, B 15X.

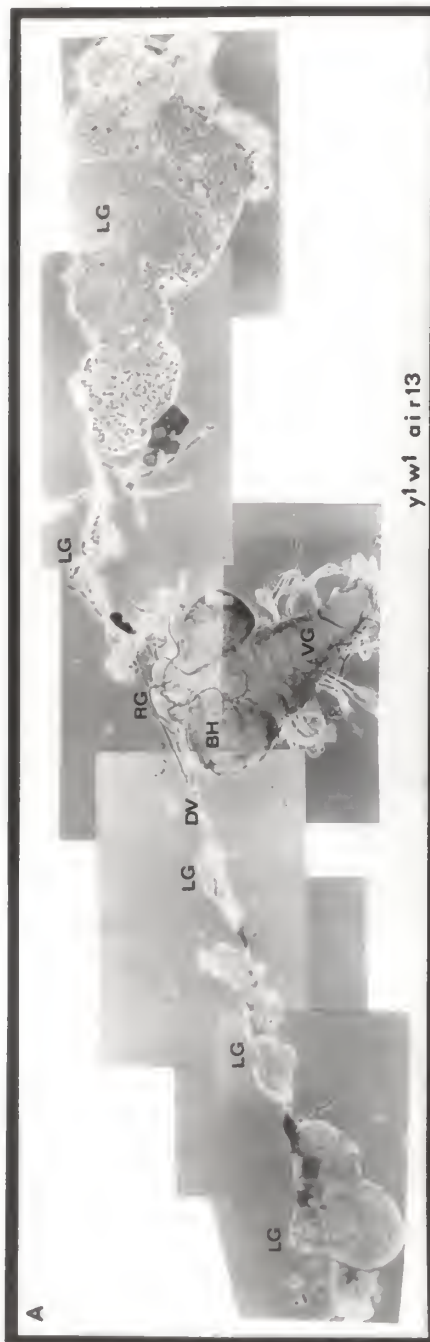


FIGURE 5.- Hypertrophy in the lymph gland lobes of air11 and air16 third instar male larvae. A, Lymph gland lobes of an air16 larva. Melanotic nodules are located in the anterior lymph gland lobes. B air16 lymph gland lobes. The insert depicts the brain complex and lymph glands of the same individual. C, Lymph gland lobes and brain-ring gland complex (insert) of an air11 larva. The anterior pair of lobes contain melanotic nodules. D, Another air11 mutant larva with hypertrophied lymph glands. Abbreviations are: BH, brain hemispheres; DV, dorsal vessel; ID, imaginal discs, LG, lymph glands; RG, ring gland; VG, ventral ganglion. Magnification A-D 27X, inserts in B and C 7X.

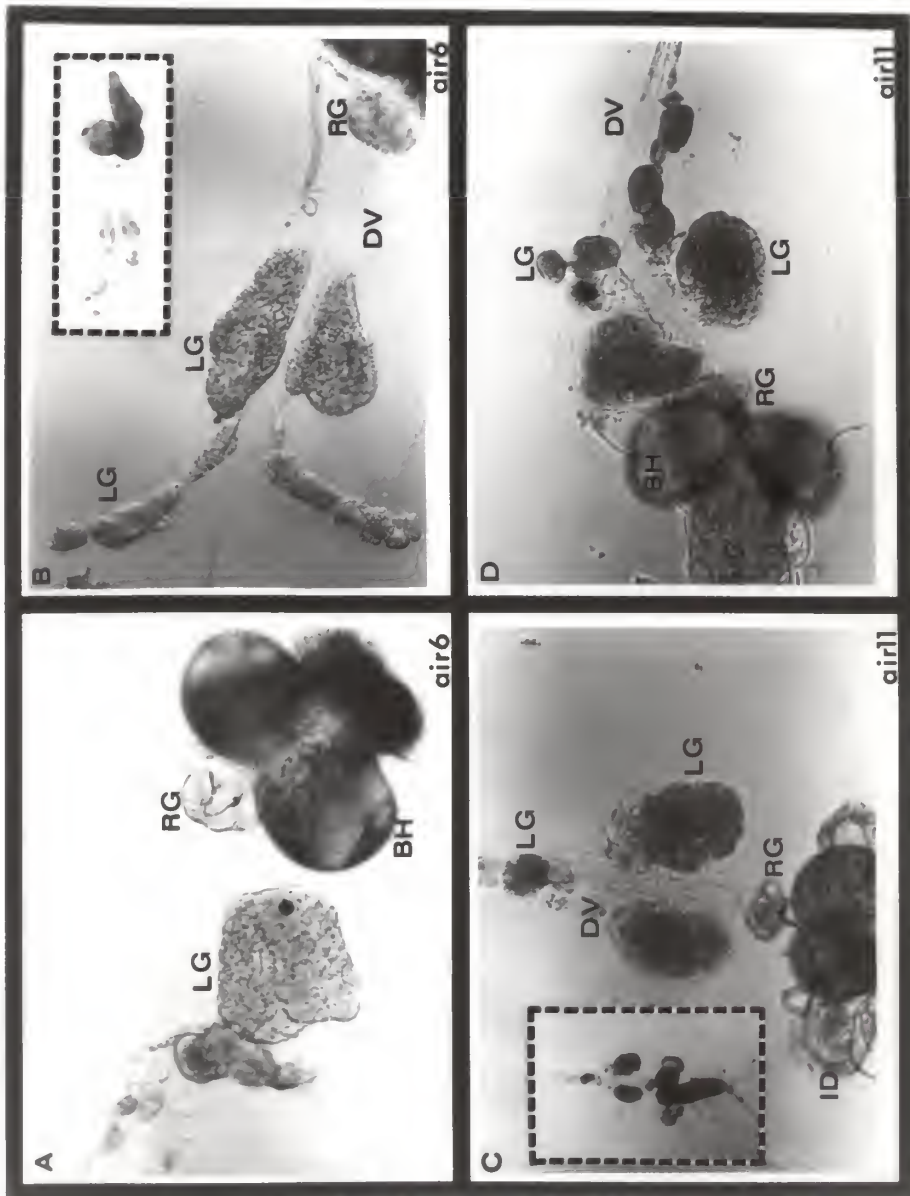
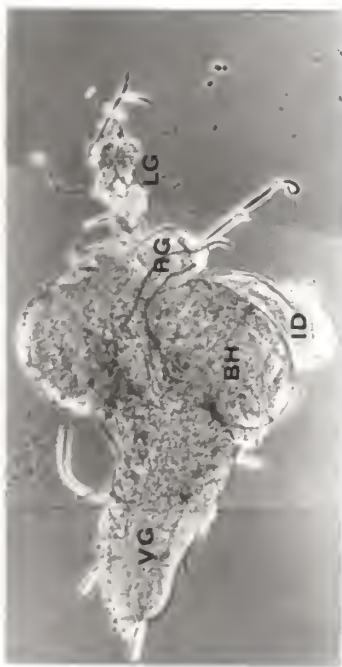


FIGURE 6.- Amplification of lymph gland lobe number in mutant versus wild type larvae. A, A montage of the brain and lymph glands in a y¹ w¹ (M strain) larva with "normal" lymph gland morphology. B, An air13 mutant displaying an increase in the number of lymph gland lobes. Abbreviations are: BH, brain hemispheres; DV, dorsal vessel; ID, imaginal discs, LG, lymph glands; RG, ring gland; VG, ventral ganglion. Magnification A 26X, B 8X.

A



y¹ w¹

B



air13

distinguishable lymph gland lobes that were enlarged slightly. It was uncertain in these mutant individuals whether the hypertrophied lobes had fragmented and adhered to the dorsal vessel or whether the lymph glands were associated with hyperplastic growth.

The portion of the alimentary canal that marks the junction between the mid- and hind-gut was melanized in the mutants: air1, air3, air4, air5, air7, air9, air10, air11, air12, air16^b and air16^c. Many imaginal disc abnormalities were observed that were subject to considerable variation between individuals bearing the same mutation. Those defects found in the majority of dissected animals were reported as characteristic of that mutant phenotype, otherwise the label "abnormal" was applied.

Observations of Melanotic Phenotypes in Non-air Stocks:
To demonstrate that the melanotic phenotype was not merely cytotype-specific or due to an endogenous mutation present in the original mutagenesis stock, an experiment was designed to examine both point lethals and deficiency stocks for the presence of melanotic larvae. The stock vials scored for the presence of tumorous larvae had been aged for a sufficient time to allow representation by all progeny classes including those classes with retarded developmental rates. Positive reports of melanotic larvae were made only when repeated examinations of these stocks revealed a significant number of larvae with melanotic

tumors or other forms of melanosis. Larvae displaying melanotic tumors were divided into two categories based on the appearance of the tumor phenotype. Category A was characterized by large, melanized tumors or strong tumor expression (Table 8A, B). Those stocks that contained third instar larvae with small melanotic tumors or some form of melanosis were assigned to Category B (Table 8A, B). These phenotypic categories were arbitrary divisions based on the severity of the tumor phenotype and do not necessarily reflect differences in immune responsiveness. The melanotic larvae observed in deficiency stocks which lacked duplications were presumed to represent the hemizygous/deficiency class of male larvae. Deficiency males maintained with Y-borne translocations must have genes/products acting in a dominant manner to give rise to melanotic tumors in these larvae. Those deficiency stocks shown to have third instar larvae with melanotic tumors at a rate higher than would be expected by "spontaneous" tumor events are listed in Table 8A. X-chromosome lethal stocks that were not induced using hybrid dysgenesis were also scored for tumorous larvae. The lethal stocks bearing tumorous larvae are listed in Table 6B. These results must be interpreted with the realization that this phenotype can exist as a normal defense response against wounding or infection and is also consistent with the presence of an unlinked mutation in the background genome.

TABLE 8A

Deficiency Stocks Containing Melanotic Larvae.

<u>Stock Description</u>	<u>Deleted Interval</u>
CATEGORY A: Large, melanotic tumors.	
Df(1)A94 / FM6	1E3;2B15
Df(1)TEM304, y ² w ⁱ ct ⁶ f/ B ^S w ⁺ y ⁺ .Y/ FM6,1	2E2-F1;3A4-6
Df(1)C149 / FM6	5A8-9;5C5-6
Df(1)ct ^{J6} , f/ C(1)DX, y w f; Dp(1;3)sn ^{13a}	6E1;7C1
Df(1)ct ^{J4} , f/ ct ⁺ Y / FM6, lethal	7A2;7C1
Df(1)v ^{L15} , y/ C(1)DX, y w f; Dp(1;2)v ^{+75d}	9B1;10A1
Df(1)DA622 / FM7	10B8;10D2
Df(1)M13 / FM7	10B10;11A3-7
CATEGORY B: Small tumors and/or Melanosis.	
Df(1)svr, spl ras ² fw/ y ² Y67g19.1/ C(1)DX, y f	1A1;1B10
Df(1)S39 / FM6	1E4;2B11-12
Df(1)w ²⁵⁸⁻⁴² / FM7	3A4-6;3C5-6
Df(1)w ^{m4L} rgt ^{3R} , w ⁻ car/ C(1)DX, y w f; Dp(1;2)w ^{+51b7}	3C1-2;3C3-4
Df(1)HA32 / ct ⁺ Y / FM6, lethal	6E4-5;7A6
Df(1)ct ⁷⁸ , y w f/ y ⁺ ct ⁺ Y/ C(1)DX, y f	6F1-2;7C1-2
Df(1)ct ^{4b1} , t ² v; Dp(1;3)sn ^{13a} (ct ⁱ)/ TM1, Me ri sbd	7B2;7C4
Df(1)RA2/ FM7	7D10;8A4-5
Df(1)KA14 / FM7	7F1-2;8C6
Df(1)C52 / FM6	8E4;9A2
Df(1)v ^{L3} / C(1)DX, y w f; Dp(1;2)v ⁺⁶³ⁱ / Cy	9F6-9;10A6-7
Df(1)RA37 / FM7c	10A6-8;10B15-17

TABLE 8B

X-Chromosome Lethal Stocks Containing Melanotic Larvae.

<u>Stock Description</u>	<u>Deleted Interval</u>
CATEGORY A: Large, melanotic tumors.	
dor ¹ / FM6	1F1;2A2
1(1)RC61 / FM7 syn: 1(1)7Ac ⁶	7A6;7A8
1(1)dsh ^{VA153} / FM7 syn: 1(1)10Bd or <u>dsh</u>	10B6;10B7
1(1)hop ^{L4} / FM7 syn: 1(1)10Be or <u>hop</u>	10B6;10B8
v 1(1)v127 fsa f ₁ / v ⁺ B ⁺ Y y ⁺ syn: 1(1)10Bj ¹	10B17;10C2
CATEGORY B: Small tumors and/or melanosis.	
1(1)HF302 / FM7 ₂ syn: 1(1)7Ac ²	7A6;7A8
1(1)GA41 / FM7 syn: 1(1)7Cg ¹	7C6;7D1
1(1)DF984 / FM7 ₁ syn: 1(1)7Ch ¹	7C6;7D1
1(1)v107 dy / v ⁺ B ⁺ Y y ⁺ / FM6 syn: 1(1)9Fh ⁴	9F4;9F8
C(1)DX, y f / y lz g 1(1)v24 / v ⁺ B ⁺ Y y ⁺	10A9
ras v 1(1)dsh f / FM7 syn: 1(1)10Bd or <u>dsh</u>	10B4;10B9
1(1)dsh ^{v26} / v ⁺ Y y ⁺ / FM7	10B6;10B7
1(1)dsh ^{M20} / FM7	10B6;10B7
ras 1(1)hop ^{v48} dy / v ⁺ B ⁺ Y y ⁺ / FM3 syn: 1(1)10Be or <u>hop</u>	10B4;10B9

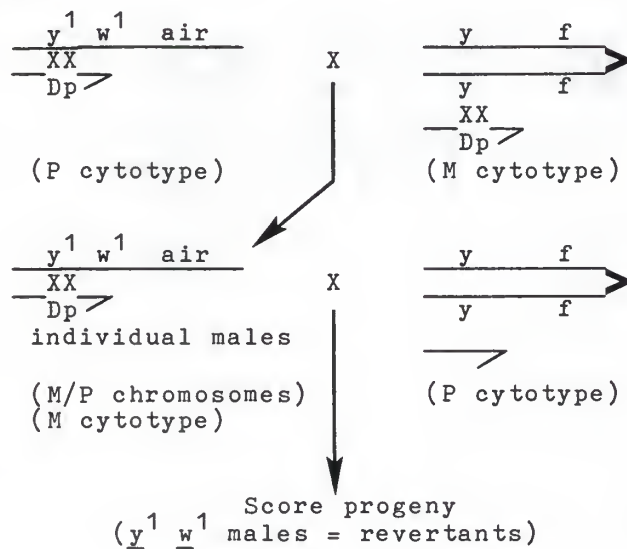
TABLE 8B

(continued)

hop ^{msv1} / FM7	10B6;10B8
1(1)hop ^{HC257} / v ⁺ Y Y ⁺ / FM7	10B6;10B8
1(1)hop ^{VA85} / v ⁺ Y Y ⁺ / FM7	10B6;10B8
1(1)hop ^{GA32} / FM7	10B6;10B8
1(1)hop ^{C111} / FM7	10B6;10B8
1(1)d.lg-1, y w sn / Binsn syn: 1(1)10Bf or <u>dlg</u>	10B8;10B9
1(1)v28 dy / v ⁺ Y Y ⁺ / FM6 syn: 1(1)10Bi ¹	10B17;10C2

Reversion of the air Mutant Lethal Phenotype: Reversion of air lethality at a rate higher than spontaneous frequencies would strongly implicate P element inserts as being responsible for the air mutant phenotype. Five air mutants that were covered with Y-borne translocations were tested for their ability to revert under dysgenic conditions (Table 9). The mutants air2, air3 and hop^{air} reverted at frequencies that were comparable or higher than 2.6-6.5% that was reported for reversion to non-lethality of P element-induced mutations in the dysgenic state (VOELKER et al. 1984). Since some P element transpositions are known to occur premeiotically (ENGELS, 1983), the high reversion frequencies observed for air2, air3 and hop^{air} stocks may be due to clusters generated by a single premeiotic event. No dysgenic revertants were obtained for air1 and air7, which might be the result of a small sample size. Since the tested mutants did not represent selected variants within the air collection, it can be assumed that the majority of air mutants contain P elements capable of transposing under dysgenic conditions.

TABLE 9

Dysgenesis-Induced Reversion of air Lethality.Scheme:Results:

Mutant	Number of Revertant males	Total Offspring	Reversion Frequency (%)
<u>air1</u>	0	8	0
	0	8	0
<u>air2</u>	19	38	50.0
	21	35	60.0
<u>air3</u>	3	26	11.5
	1	10	10.0
<u>air7</u>	0	7	0
	0	4	0
	0	11	0
	0	6	0
<u>hop</u> ^{air}	1	19	5.3

DISCUSSION

Cellular immunity in Drosophila involves a series of integrated reactions leading to the encapsulation of foreign objects that are too large to be phagocytosed. The reactions include: recognition of "non-self", developmentally-regulated transformation of plasmatocytes to lamellocytes, aggregation and layering of haemocytes to form a cellular capsule, and the deposition of melanin to render the immune capsule and its contents physiologically inert (CHADWICK, 1975; GATEFF, 1984; GOTZ, 1986; NAPPI, 1975; RIZKI, 1978). It was suggested that tumorigenesis (haemocytic encapsulation) in Drosophila was under the control of two polygenic systems (CORWIN and HANRATTY, 1976). The two systems were involved in regulation of haemocyte differentiation and transformation and in reactions leading to the initiation of melanization. Since genetic control of the cellular defense reactions could operate at many steps in this process, it is not surprising that great variability in the expression and penetrance of the tumorous phenotype exists in the known melanotic tumor mutants (SPARROW, 1978). The assessment of variable expression and penetrance of the tumor phenotype usually is based only on the appearance of melanotic tumors and not on the presence or absence of the haemocytic capsule. RIZKI and RIZKI (1986) have demonstrated that amelanotic capsules

are formed in some tumor mutants supporting the hypothesis that the deposition of melanin is under different regulatory control than the encapsulation reactions. In addition, melanin formation is closely associated with a variety of physiological reactions involving phenoloxidase enzymes that require careful regulation to avoid exposing the larvae to the toxic intermediates generated in these reactions (SODERHALL and SMITH). It may well be that fluctuations in the deposition of melanin are responsible for most of the variability in expression and penetrance that is associated with the melanotic tumor phenotype.

The air mutations described in these studies have genetic lesions which trigger some form of autoimmune response leading ultimately to the development of melanotic tumors. It was important to determine whether the lethal and melanotic tumor phenotypes expressed by the air mutants were the consequence of a single mutational event or due to multiple mutational events. We attempted to separate tumor formation from the lethal phenotype by examining third instar male larvae bearing: 1) non-lethal recombinant chromosomes containing material which flanked but did not include the lethal air locus 2) dysgenically-induced, non-lethal revertant air chromosomes and 3) duplication-covered air chromosomes. All strategies failed to separate the lethal and tumorous aspects of the air mutant phenotype suggesting that both traits were caused by a single genetic

lesion. The phenotype of the X-linked, neoplastic mutant tumorous-lethal (Tum¹) consists of many distinct pleiotropic effects including lethality, lymph gland neoplasia and a melanotic tumor phenotype (HANRATTY and RYERSE, 1981). Experiments that were designed to separate the lethal and tumor phenotypes in the Tum¹ mutant failed to reveal any evidence that the two characteristics assorted or segregated independently. Both Tum¹ and another well-characterized, X-linked tumor mutation tu(1)Sz^{ts} exhibit a temperature-sensitive, tumor phenotype. Unlike these mutations, the air mutants did not show a temperature-sensitive adult tumor phenotype (data, not shown), and any temperature-influenced effects on larval pathology were not investigated. Heterozygous Tum¹ females at the restrictive temperature express a dominant melanotic tumor phenotype in both larvae and adults. We have noticed dominant melanotic tumor expression in heterozygous larvae from many of the air mutant stocks.

The 20, P element-induced air mutations resolved into a minimum of 14 separate complementation groups that were distributed along the length of the X-chromosome. Confirmation of additional allelic relationships between air mutants covered by a common duplication, was not possible due to the infertility of some duplication-covered air males. Three of the air loci had additional allelic variants associated with them, namely: the complementation

group at 1(1)7Ac (LINDSLEY and ZIMM, 1987), hopscotch (hop, PERRIMON and MAHOWALD, 1986) and air16 (this study). The lethal period associated with each air mutant was multi-phasic suggesting that the wild type gene products of the air loci may be required at many distinct developmental steps or continuously throughout larval development. The multi-phasic lethality might indicate that air products are required early in development, and mutant products will induce melanotic tumor formation if the individual survives to later stages of development.

It was interesting to note that stocks bearing X-chromosome deficiencies, whose cytological limits included air loci, frequently contained tumorous larvae. Larvae from the deficiency stocks Df(1)TEM304, Df(1)DA622 and Df(1)M13 exhibited melanotic tumors characteristic of Category A (Table 8A, B) or a strong melanotic tumor phenotype, but these deficiencies did not include any of the air mutant loci. However, the melanotic tumor mutant, 1(1)ml was previously mapped to region that falls within the limits defined by Df(1)TEM304 (LINDSLEY and GRELL, 1968). The other two deficiency stocks may uncover additional loci in which a loss-of-function leads to melanotic tumor formation. Another dysgenesis-induced lethal with an air mutant phenotype was mapped within the boundaries of Df(1)A94 but was not allelic to air2 suggesting the existence of additional tumorous loci (A. L.

Johnson, unpublished results). This explanation is supported by the high correlation that existed between tumorous larvae observed in lethal point mutant stocks (not previously associated with a melanotic phenotype) and those observed in deficiency stocks that included the point mutant loci within their cytological boundaries. For example, the deficiency Df(1)DA622 includes l(1)disc-large (l(1)d1g) (PERRIMON and MAHOWALD, 1986) and the deficiency Df(1)M13 includes both l(1)10Bi¹ and l(1)10Bj¹ (LINDSLEY AND ZIMM, 1987). All of these deficiency and lethal point mutant stocks contained tumorous larvae. The presence of larvae with a strong melanotic tumor phenotype was noted in the Df(1)v^{L15} stock and this deficiency fails to complement the melanotic tumor mutant air12. All of the deficiency stocks that exhibited small tumors or some other form of melanosis (Category B, Table 8) also contained melanotic tumor loci within their deficiency interval except the following: Df(1)S39, Df(1)w²⁵⁸⁻⁴², Df(1)w^{m4L} and Df(1)HA32. We expect that these deficiency stocks include unidentified tumor genes or genes which are capable of influencing tumor formation at other loci. Although the melanotic tumor phenotype could have been caused by mutations at different sites in the genome it seems unlikely that there would be such a high correlation between lethal point mutant stocks bearing tumorous larvae, the air mutant loci and allelic deficiency stocks that also

give tumorous larvae.

Various lethal point mutant stocks bore tumorous larvae (Table 8B), including two alleles of air7, seven variants of the hopscotch locus, and four dishevelled (dsh) alleles. Developmental and genetic analyses of the hop locus have indicated that products of this locus are required both maternally for proper embryonic development and zygotically for the proper regulation of cell division in late larval stages (PERRIMON and MAHOWALD, 1986). Mutations of the dsh locus are associated with extreme segment polarity defects in developing embryos, presumably caused by the inability of the mutant embryos to maintain or express a determined state (PERRIMON and MAHOWALD, 1987). The dsh gene product was also required late in larval development for the proper differentiation of imaginal discs (PERRIMON and MAHOWALD, 1987). It is interesting that mutations at these two loci were associated with tumor expression during the late larval stage, which coincided with the time that the wild type products of these loci are required to complete proper development.

We have cytologically mapped the melanotic tumor mutation Tum¹ to 10A1;10A6-7 which is a region reported to contain approximately four distinct complementation groups (GEER, LISCHWE and MURPHY, 1983; ZHIMULEV et al. 1981). This map position was derived from coverage by the duplications v⁺y y⁺3 (9F3;10E3-4) and v y y⁺ (9F3;10C1-2) and exclusion from

the deficiencies Df(1)v^{L15}, Df(1)RA37, Df(1)KA7, Df(1)N71, and Df(1)M13. It was noted that Tum¹ females in heterozygous combination with chromosomes containing the genetic variants: hop^{air}, hop^{v48}, dlg, l(1)v28 (l(1)10Bi¹), l(1)V178 (l(1)10Bb¹², LINDSLEY and ZIMM, 1987), Df(1)v^{L15}, Df(1)v^{L2}, Df(1)RA37, Df(1)KA7, Df(1)N71, Df(1)DA622 and Df(1)N12 showed enhanced melanotic tumor expression at the restrictive temperature. Since the Tum¹ mutation is associated with a dominant tumor phenotype in females at 29°C (NAPPI and CARTON, 1986), the enhanced tumor expression was assessed by comparing Tum¹/deficiency (or Tum¹/lethal) females to Tum¹/balancer sibling females generated from the same cross. The non-lethal melanotic tumor mutant tu(1)Sz^{ts} (RIZKI and RIZKI, 1980b) when reared at the restrictive temperature exhibited enhanced tumor expression in heterozygous combination with Df(1)v^{L15}, Df(1)v^{L3}, Df(1)v^{L2}, the deficiency segregant of T(1;2)v^{65b}, Df(1)DA622, Df(1)KA7, l(1)Q54 (l(1)9Fe¹, LINDSLEY and ZIMM, 1987) and hop^{v48}. The tu-Sz^{ts} mutation was previously localized to 10A10-10A11 (RIZKI and RIZKI, 1980b) and is not allelic Tum¹ based on our cytological map position (10A1; 10A6-7). These results strongly suggest that some melanotic tumor loci can interact synergistically or quantitatively with other genes to produce a tumor phenotype.

We propose that melanotic tumor mutations can be

classified into two general groups based on how the immune responses are triggered. The first group includes those genetic lesions which cause abnormal cell surface components to be expressed or presented to the immune surveillance system. The second group is represented by mutations which non-specifically induce defense responses because of the abnormal temporal or spatial distribution of haemocytes (ie. lymph gland neoplasia; HANRATTY and RYERSE, 1981). RIZKI and co-workers (RIZKI and RIZKI, 1974a, b; RIZKI and RIZKI, 1980a, b) have demonstrated that the specific encapsulation of caudal adipose cells in the tu-W and tu-Sz^{ts} mutants that coincides with basement membrane degeneration in the fat body tissue. This mutation is representative of the first group of melanotic tumor mutant, in which a specific tissue defect induces the defense response. Recent studies by KNIEBIEHLER et al. (1987) have ascribed a role for haemocytes in basement membrane deposition based on the production and accumulation of type IV collagen gene transcripts by the haemocytes. Prior to these studies EL SHATOORY (1957a,b,c) had reported that a relationship existed between the cycles of hypertrophy and regeneration occurring in the lymph glands at each larval molt, and the proper differentiation of the testes, hind-gut primordia, proventriculus and the salivary gland primordia. Migratory cells released from the lymph glands (presumably the haemocytes) were the

mediators in the differentiation or morphogenesis of these developing tissues. It is still unclear what role the haemocytes may play in various morphogenetic or differentiation processes.

We tried to determine whether specific developmental abnormalities might have triggered the immune response system in the air mutants. The rationale for this study was to identify specific morphological defects observed in mutant larvae and correlate these with the sites of melanotic encapsulation. We used two approaches 1) larval anatomy was scored for the presence of developmental defects or melanization and tumor formation and 2) the lymph glands were examined specifically for any evidence of hypertrophy that might suggest neoplastic growth. The first approach allowed us to identify some consistent anatomical anomalies that were sometimes associated with melanotic tumor formation and/or melanosis. Melanotic tumors or melanosis were observed only infrequently in some air mutants due to the variability that existed in expression and penetrance of the melanotic tumor phenotype. One feature common to all of the air mutants was a small brain phenotype. Although the morphology of the brain was often abnormal the small size applied to both the formation centers of the optic lobes and the ventral ganglion. The ring gland in most of the air mutant larvae examined was proportional to the size of the brain. In mutants

containing ring glands that approached the size proportions expected for wild type larvae, the ring glands were abnormally thin and diffuse in appearance. Defects in the ring gland may have been responsible for the extended larval life observed in some of the air larvae. A portion of the intestine which corresponded to the hind-gut imaginal ring (BRYANT and LEVINSON, 1985), was frequently melanized in the mutants: air1, air3, air4, air5, air7, air9, air10, air11, air12, air16^b and air16^c. Melanosis in a region of the proventriculus that may represent the fore-gut imaginal ring was observed in air6 mutant larvae. The imaginal primordia of the gut (imaginal rings), are diploid cells found in larvae that grow by cell proliferation until metamorphosis, at which time point they give rise to portions of the adult intestine (BRYANT and LEVINSON, 1985). Although abnormal proliferation in the imaginal ring cells had been reported for some mutants with extended larval periods (BRYANT and LEVINSON, 1985), we found no evidence that air mutants with prolonged larval periods were more likely to exhibit melanized imaginal rings. It may be significant that the observed gut melanizations were confined to the imaginal ring centers, which are one of the few cell populations in a larva that retains the ability to divide.

Another interesting characteristic associated with the mutants air8 and air16^b was the presence of melanotic

garland cells (wreath cells). The garland cells are found in a clumped mass at the junction between the oesophagus and the proventriculus and they function to remove toxic wastes from the haemolymph (RIZKI, 1978). The observed melanization in the garland cells may have been caused by the absorption and/or accumulation of melanin obtained from the haemolymph. This may imply that abnormal humoral reactions created these melanotic products, perhaps in response to an inappropriate signal caused by the air mutations.

The second approach that was used to identify specific developmental defects associated with the air mutant phenotype was the examination of mutant male lymph glands. This analysis revealed two types of lymph gland defects. One defect was the extreme hypertrophy of lymph gland lobes that often resulted in rupture and dispersal of the lymph gland fragments throughout the haemocoel (not an artefact of dissection). The mutants: air2, air6, air7, air8, air9, air11, air13 and air15 showed varying degrees of such lymph gland hypertrophy, with small melanotic nodules found in the majority of hypertrophied lymph gland lobes. Total melanotic encapsulation of some lymph gland lobes was observed in many of these mutants. Confirmation that the free-floating masses found in the haemocoel were primarily of lymph gland origin came from observations of incompletely melanized capsules. This was an extremely

interesting observation since lymph gland hypertrophy in other mutants has been associated with a transplantable neoplasm of this gland (ie. Tum¹, HANRATTY and RYERSE, 1981; NAPPI and CARTON, 1986). Some air mutant individuals also displayed variability in the size of the imaginal discs, ring gland and other structures suggesting that there were more global defects in growth regulation.

The other lymph gland defect that was observed in air13 was an amplification in the total number of lymph gland lobes. This phenotype also was found in conjunction with lymph gland hypertrophy. Lymph glands normally consist of 4-6 pairs of lobes with a predictable morphology that is dependent on the position that the lobes occupy along the length of the dorsal vessel (SHRESTHA and GATEFF, 1982). The air13 mutant lymph glands were observed with up to 16 separate lobes of variable sizes that were independent of their position along the dorsal vessel. These lobes also were larger than wild type lymph gland lobes.

The present study has provided evidence that at least 14, distinct X-chromosome loci can be mutated to give a lethal melanotic tumor phenotype. We have made a preliminary characterization of the mutant syndrome associated with each aberrant immune response (air) mutation and identified a number of developmental defects that may contribute to the melanotic tumor phenotype. Based on these preliminary observations, the air mutations appear to be equally

distributed between the proposed classes of melanotic tumor mutants. The consistent melanization in cells or tissues caused by some air mutations, suggests that a specific defect in these cells/tissues triggers melanotic tumor formation (Class 1). Eight air loci were associated with hypertrophy of the lymph gland lobes in mutant male larvae. These eight air mutations may contain neoplastic or abnormal functioning lymph glands that initiate melanotic tumor formation (Class 2).

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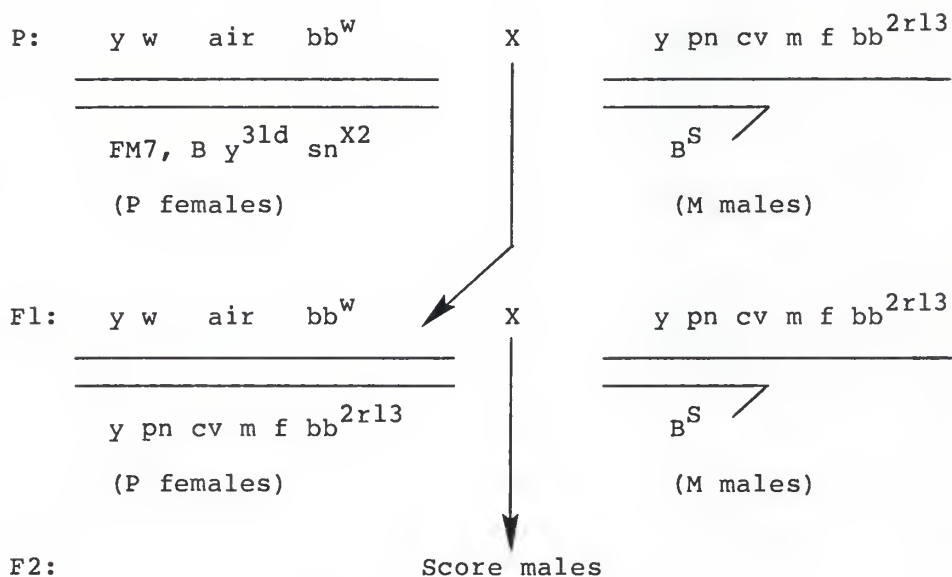
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Appendix 2: Recombination Mapping--air Lethals

(y pn cv m f bb^{2rl3}).

Mapping Stock: y pn cv m f bb^{2rl3}



Marker Mutations:

air	=	X	lethal, melanotic tumor mutation
y	=	0.0	yellow
pn	=	0.8	prune
w	=	1.5	white
cv	=	13.7	crossveinless
sn	=	21.0	singed
m	=	36.1	miniature
f	=	56.7	forked
B _S	=	57.0	Bar
B _S ^w	=	57.0	Bar of Stone
bb ^w	=	66.0	bobbed (weak)
bb ^{2rl3}	=	66.0	bobbed (lethal allele)
FM7	=	X	Balancer Chromosome
P	=		P cytotype
M	=		M cytotype

Appendix 3: Construction of an Additional Mapping Stock:
sc ec cv ct v m f.

<u>Mapping Stock a</u>	<u>Mapping Stock b</u>
<u>sc ec cv ct v g f</u>	<u>C(1)DX / B^S Y</u>
1, FM7(w ^a B sn ^{X2} y v g)	y pn cv m f bb ^{2rl3}

1st Cross:

Pl:

<u>sc ec cv ct v g f</u>	X	<u>y pn cv m f bb^{2rl3}</u>
		B ^S /
1, FM7		(males)
(B, g, females)		

2nd Cross:

<u>sc ec cv ct v g f</u>	X	<u>sc ec cv ct v g f</u>
		Y /
y ⁺ pn cv m bb ^{2rl3}		(males)
(y ⁺ cv f females)		
Collect non-bobbed males:		<u>sc ec cv ct v m f</u>
		Y /

3rd Cross: Cross males to attached-X females to maintain the X-chromosome in males.

4th Cross: Recombination Mapping Crosses.

<u>sc ec cv ct v m f</u>	X	<u>air</u>
Y /		FM7 (B)
<u>sc ec cv ct v m f</u>	X	<u>sc ec cv ct v m f</u>
air		Y /

SCORE MALES

Selected Mapping Stock Generated Using this Protocol:

sc ec cv ct v m f / C(1)DX, y w f
 92

Appendix 4: Recombination Mapping Results--air Lethals

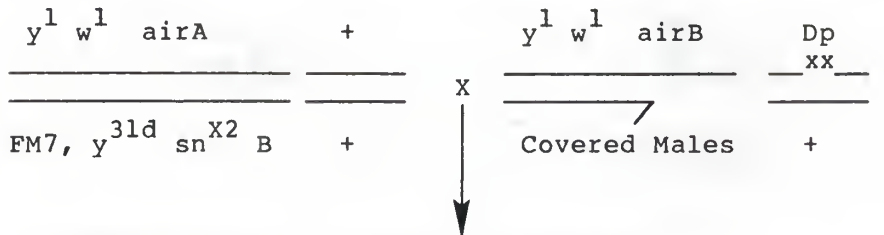
Mutant	Mapping Stock	Marker Interval	Total males Scored	Map Position
<u>air1</u>	1	sc-w	491	0.5
	2	pn-cv	376	2.1
<u>air2</u>	1	w -ec	72	2.8
	2	pn-cv	276	4.3
<u>air3</u>	1	sc-w	213	1.0
	2	w -cv	121	1.7
<u>air4</u>	2	cv-m	529	13.9
<u>air5</u>	2	cv-m	277	15.6
<u>air6</u>	3	cv-ct	104	15.0
<u>air7</u>	2	cv-m	308	15.9
<u>air8</u>	2	cv-m	426	22.3
<u>air9</u>	2	cv-m	125	20.0
<u>air10</u>	2	cv-m	110	21.9
<u>air11</u>	2	cv-m	153	29.0
<u>air12</u>	2	cv-m	246	33.4
<u>air13</u>	2	cv-m	259	35.4
<u>hop</u> ^{air}	2	cv-m	227	35.3
<u>air14</u>	3	m -f	107	44.9
<u>air15</u>	2	m -f	110	55.6
<u>air16</u> ^a	2	m -f -+	296	56.2
<u>air16</u> ^b	3	m -f -+	219	56.7
<u>air16</u> ^c	3	m -f -+	105	56.7
<u>air16</u> ^d	2	m -f -+	288	56.7

1 = sc ec cv ct v g f 2 = y pn cv m f bb^{2rl3}

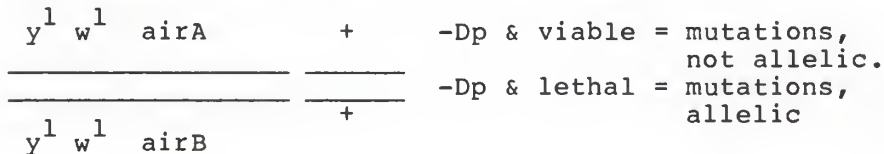
3 = sc ec cv ct v m f

Appendix 6: air Lethals--Allelism Tests

(1) Covered Males with Autosomal-Borne Duplications:

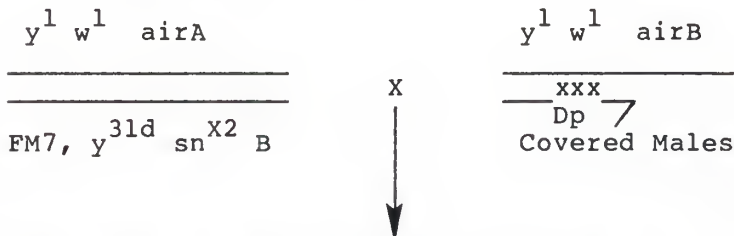


F1 females bearing lethal chromosomes:

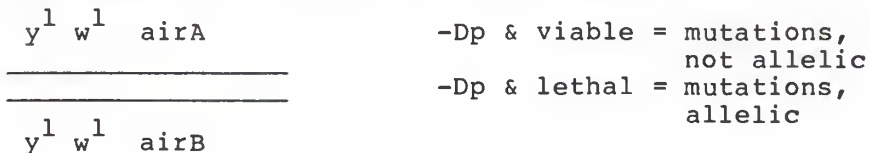


NB: The homologous autosome is usually marked.

(2) Covered Males with Y-Borne Duplications:



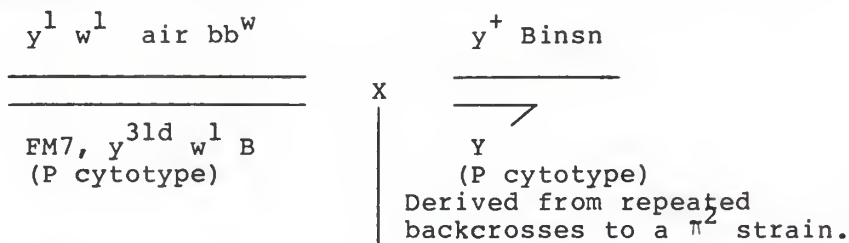
F1 females bearing lethal chromosomes:



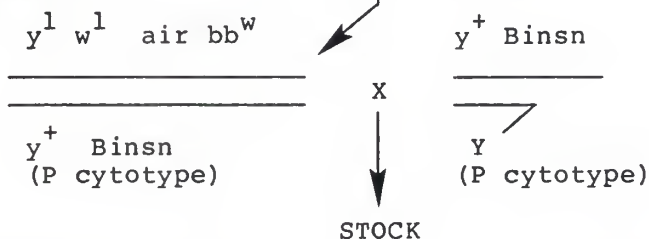
A, B = two different air mutants.

Appendix 7: air Lethals Placed Over a Binsn Balancer

Cross 1:



Cross 2:



Larval Mouthparts in Stock (P cytotype):

y^+ Binsn	larvae = black mouthparts (y^+ / y^+)
<hr/>	
<hr/>	
y^+ Binsn	
<hr/>	
y^+ Binsn	larvae = black mouthparts (y^+)
<hr/>	
y	
<hr/>	
$y^1 w^1 \text{ air } bb^w$	larvae = black mouthparts (y^+ / y^1)
<hr/>	
y^+ Binsn	
<hr/>	
$y^1 w^1 \text{ air } bb^w$	larvae = brown mouthparts (y^1)
<hr/>	
y	

Appendix 8: Analysis of Recombinant Males that Contain Flanking X-Chromosome Material.

<u>y w air bb^w</u>	X	<u>y pn cv m f bb^{2rl3}</u>
y pn cv m f bb ^{2rl3} (M/P chromosomes)	↓	B ^S / (M chromosomes)

Recombinant males containing markers which flank the presumed lethal locus were collected and mated to attached-X females (M cytotype).

Vials of third instar larvae were scored for the presence or absence of melanotic tumors.

Results

Mutant	Recombinant air Chromosome Constitution	# Vials Examined	# Vials with Tumors *
--------	---	------------------	-----------------------

<u>air1</u>	y pn cv ⁺ m ⁺ y w cv ⁺ m ⁺	8 6	0 2
<u>air2</u>	y w cv ⁺ m f y w cv ⁺	8 6	0 6
<u>air4</u>	y pn cv m ⁺	8	4
<u>air7</u>	cv m ⁺	8	4
<u>air8</u>	y pn cv ⁺ m ⁺ y w cv ⁺ m	4 8	4 0
<u>air9</u>	y pn cv ⁺ m ⁺ f ⁺ y w cv ⁺ m f y w cv m f	8 8 8	0 0 0
<u>air10</u>	y w cv ⁺ m ⁺ cv m ⁺	8 4	0 2
<u>air11</u>	y pn cv ⁺ m ⁺ y w cv ⁺ m	8 4	0 2
<u>air13</u>	cv ⁺ m ⁺ cv ⁺ m ⁺	8 8	0 0
<u>hop</u> ^{air}	cv ⁺ m ⁺ cv ⁺ m	8 8	0 0

Appendix 8: (continued)

<u>air15</u>	y	w	cv ⁺	m ⁺	f	8	0
	y pn		cv ⁺	m ⁺	f	8	0
<u>air16</u> ^d				m ⁺	f	6	2
Mapping Stock (M cytotype):							
	y pn		cv	m	f bb ²	8	0
y ¹ w ¹ Stock (M cytotype):							
	y ¹	w ¹			bb ^w	6	4

* = The tumors observed in these experiments consisted of small-medium sized, light brown pigmented material located at the anterior junction of the mid- and hind-intestine. In addition, small light brown pigmented nodules were found lining the dorsal vessel. The melanosis observed in these stocks was distinctly different from the tumor phenotype associated with the air mutations.

Appendix 9: Duplication/Deficiency Results--air Lethals.

Duplication Name (Breakpoints)		air11	air13	air14	air15	air16 ^d
sn ^{+72d}	(7A8;8A5)	-	-			
v ^{+75d}	(9A2;10C2)	-	-		-	
v ⁺⁶³ⁱ	(9E11;10A11)	-	-		-	-
v ^{+Yy} ⁺	(9F3;10C1-2)	-	-	-	-	
v ^{+Yy} ⁺³	(9F3;10E3-4)	-	-	-	-	
v ^{65b}	(10A1;11A7)	-	-	-	-	
r ^{+f} ⁺	(13F10;16A1-2)	-	-	-	-	-
r ^{+75c}	(14B13;15A9)	-	-	-	-	-
f ^{+71b}	(15A4;16C2-3)	-	-	-	-	+/-
Deficiency Name (Breakpoints)						
v ^{L15}	(9B1;10A1)	-	-		-	
v ^{L3}	(9F6-9;10A6-7)	-			-	-
v ^{L2}	(9F13;10A1)	-	-			
KA7	(10A9;10F10)	-	-	-	-	
N71	(10B5;10D4)	-	-		-	
DA622	(10B8;10D2)			-	-	
sd ^{72b26}	(13F1;14B1)				-	-
D15	(14D1;15C5)	-	-	-	-	-
D17	(14F6;15A6)				-	-

- = complements ie. not allelic.

+ = fails to complement ie. allelic.

P Element-Induced, X-Linked Lethal Mutations Causing
Melanotic Tumors in Drosophila melanogaster

by

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AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

Twenty, X-linked lethal mutations exhibiting melanotic tumor phenotypes were isolated in a P element mutagenesis experiment. These aberrant immune response (air) mutations resolved into 14 separate complementation groups that were distributed along the X-chromosome. Alleles of the hopscotch locus and the 1(1)7Ac complementation group were represented in the air mutant collection. The lethal period associated with each air mutation was multi-phasic with lethality occurring predominantly at the late larval-early pupal boundary. The air mutant, lethal syndrome included a small brain phenotype in addition to other morphological defects which may have contributed to the observed melanotic tumor phenotype. Seven air mutations gave rise to hypertrophied lymph glands (hematopoietic organs) that are characteristic of mutants with invasive lymph gland neoplasms. The phenotype expressed by the air mutants is consistent with the suggestion that abnormal cellular immune responses are implicated in melanotic tumor formation